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# SOIL SCIENCE

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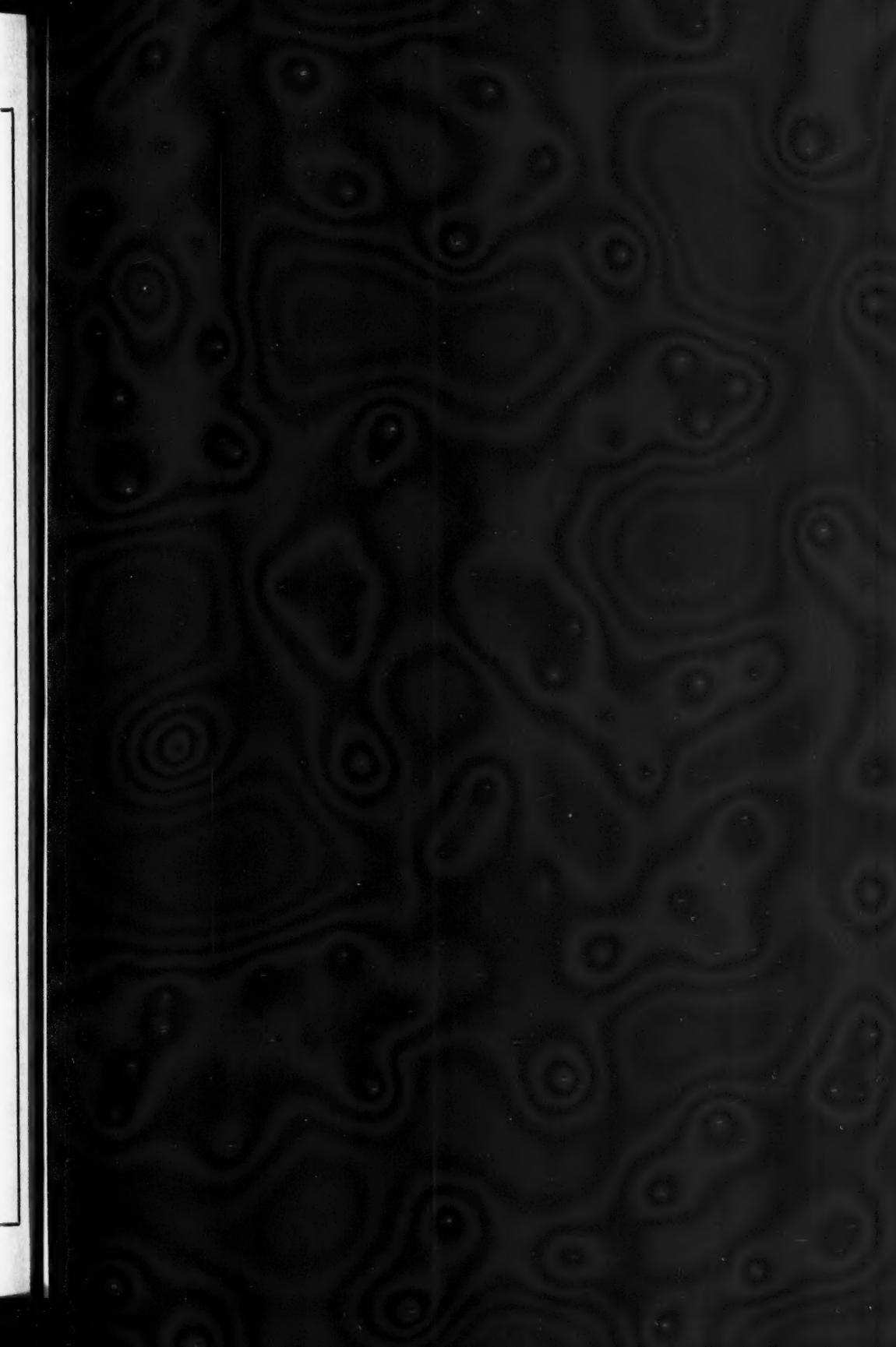
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## THE COMPOSITION OF BIENNIAL WHITE SWEET CLOVER AS RELATED TO SOIL ENRICHMENT

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*Illinois Agricultural Experiment Station*

Received for publication November 13, 1925

The increasing use of the various sweet clovers, especially the biennial white variety, for soil enrichment and for other purposes, all of which are eventually related to soil fertility, suggested the need for more information on the chemical composition of this crop. The purpose of this study was to determine the composition of the tops and of the roots at various stages of growth, in order more accurately to advise how to handle this clover in the various cropping systems.

The literature contained no systematic studies dealing with the composition of sweet clover, with the exception of some nitrogen analyses. Since the completion of this work, Willard (4) has published data from field experiments on the nitrogen content and yield of tops, stubble, and roots of the biennial white sweet clover, when seeded with oats.

The samples analyzed in the work reported here were collected from a limited area seeded for the purpose of this study. The soil type on which the samples grew was a brown silt loam, the surface of which was slightly acid, and the subsurface and subsoil of which contained limestone particles. Successive adjacent samples were taken. Every precaution was taken to obtain uniformly developed plants. The root samples were taken 14 inches deep. The tops were separated from the roots at the time of collection. Duplicate analyses were made on all samples. The chemical methods employed were the recognized methods for plants: the total nitrogen method included nitrates; and sulfur was determined by the bomb method. In most cases about thirty plants constituted the sample, although the one of October 15 contained one hundred plants. All figures are reported on the water-free basis. The results are given in pounds per ton and on a percentage basis in separate tables, for convenience in comparison with other similar data.

### COMPOSITION OF TOPS AND ROOTS, GROWTH OF FIRST SEASON

The composition of the fall growth of tops and roots, which was determined first, is presented in tables 1 and 2. The complete data appear in table 5.

<sup>1</sup>This work was conducted while the authors were on the staff of the Agronomy Department of the University of Illinois. The senior author is now connected with the University of Wisconsin, and the junior author is on the Ohio State University staff.

The tops decrease in nitrogen, phosphorus, sulfur, and potassium, but change very little in calcium and magnesium content. One sample is out of line with

TABLE 1  
*Changes in composition of sweet clover tops (first season's growth)*

Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1919</i>						
July 25.....	62.40	4.88	12.48	31.46	30.36	11.08
September 10.....	60.80	3.10	10.56	25.82	30.56	9.56
October 10.....	55.80	2.26	10.28	19.46	32.66	11.34
October 15.....	47.00	2.22	10.10	21.82	28.36	9.94
October 25.....	43.00	2.16	8.90	15.88	29.76	10.00
November 1.....	56.00	5.94	8.10	32.74	38.58	7.66
December 2.....	30.00	0.92	5.56	6.64	18.14	6.80

TABLE 2  
*Changes in composition of sweet clover roots (first season's growth)*

Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1919</i>						
October 15.....	61.60	5.00	4.58	12.14	6.14	5.66
October 25.....	59.80	5.84	5.06	14.40	6.26	5.18
November 1.....	71.40	8.92	3.84	22.16	6.54	4.48
December 31.....	66.20	4.80	5.48	15.26	5.86	3.74

TABLE 3  
*Changes in composition of sweet clover tops (second season's growth).*

Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1920</i>						
May 10.....	82	5.64	10.24	27.74	31.10	13.58
May 20.....	62	3.95	9.44	26.68	26.04	10.04
June 3.....	49	3.56	8.46	37.54	23.64	9.46
June 19.....	33.4	2.50	7.82	23.94	14.56	8.64
July 3.....	34.2	2.46	6.86	16.46	17.32	9.22
July 17.....	36	2.30	6.20	15.60	17.08	8.04
August 1.....	36	2.16	5.70	13.92	18.96	7.46
August 14.....	24.4	1.48	5.34	12.74	14.82	5.40
August 28.....	26	1.16	4.00	20.04	11.60	4.66
September 11.....	27.2	1.28	3.98	12.96	7.80	3.96
October 1.....	26	1.50	3.58	11.70	9.46	4.36

the others for some unknown reason, although special attention was given this sample to determine the cause of its higher content of nitrogen, phosphorus,

potassium, and calcium. No evidence of irregular soil condition could be obtained, and the only tenable assumption seemed to be that it had remewed its growth of tops because of favorable weather conditions. The high calcium and phosphorus content suggested the presence of a phosphate in the area of the soils from which this sample was taken. Similar difficulties in composition studies in successive sampling have been reported by other workers.

The loss in nitrogen, phosphorus, sulfur, and potassium in the tops is gradual and more regular than would be accounted for by the action of rain. As care was exercised to avoid loss of leaves no change in composition can be attributed to that possibility. This decrease was not accompanied by an increase in dry matter, except on the earliest dates. The calcium and magnesium decreased very slightly and then only at the last sampling, disregarding the

TABLE 4  
*Changes in composition in sweet clover roots (second season's growth)*  
Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1920</i>						
March 20.....	70.2	5.06	6.00	11.00	6.00	5.54
April 24.....	73.8	5.16	8.26	10.82	10.32	8.02
May 10.....	71.4	3.66	8.50	11.50	9.32	7.24
May 20.....	57.4	2.30	7.74	11.12	9.74	7.54
June 3.....	43.6	2.28	8.64	11.18	9.32	7.86
June 19.....	40.0	2.20	7.66	19.94	8.66	8.56
July 3.....	33.0	1.92	6.04	12.68	8.54	8.40
July 17.....	24.0	1.80	6.10	16.42	9.00	8.40
August 1.....	27.4	1.66	5.94	19.82	9.40	8.38
August 14.....	27.2	1.46	5.86	19.08	10.66	8.34
August 28.....	21.6	1.12	5.76	31.48	11.76	6.98
September 11.....	15.6	1.36	4.06	21.72	8.54	7.06
October 1.....	18.4	1.90	3.86	21.00	10.66	6.32

November 1 sample. One would predict that if a crop is to store elements for the next season's growth, nitrogen, phosphorus, and sulfur would be the elements most concerned in the process.

An examination of the root composition shows larger amounts of nitrogen and phosphorus than those found in the tops. It is also noted that the roots gained in these elements at the same time that they increased in weight of dry matter. The roots are much richer in nitrogen and phosphorus than the tops of the same plants on the same dates. This is not true of the other elements. From this fact, one may assume that the plant stores nitrogen and phosphorus, but does not translocate all the mineral elements, except in a very limited way. The data do not prove a translocation of the material from the tops to the roots, but suggest it very strongly for nitrogen and phosphorus and slightly for sulfur. The roots are richest in nitrogen and potassium,

followed by calcium and then by phosphorus, sulfur, and magnesium; among the last three there is little difference.

TABLE 5  
*Composition of sweet clover tops and roots*  
Percentages on water-free basis

DATE	PART OF PLANT	PROPORTION OF WHOLE PLANT <i>per cent</i>	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1919</i>								
July 25.....	Tops	3.12	0.244	0.624	1.573	1.518	0.554	
September 10.....	Tops	3.03	0.155	0.528	1.291	1.528	0.478	
October 10.....	Tops	2.79	0.113	0.514	0.973	1.633	0.567	
October 15.....	Tops	42	2.35	0.111	0.505	1.091	1.418	0.497
October 15.....	Roots	58	3.08	0.250	0.229	0.607	0.307	0.283
Octobet 25.....	Tops	37	2.15	0.108	0.445	0.794	1.488	0.500
October 25.....	Roots	63	2.99	0.292	0.253	0.720	0.313	0.259
November 1.....	Tops	..	2.80	0.297	0.405	1.637	1.929	0.383
November 1.....	Roots	..	3.57	0.446	0.192	1.108	0.327	0.224
December 2.....	Tops	..	1.50	0.046	0.228	0.332	0.907	0.340
December 31.....	Roots	..	3.31	0.240	0.274	0.763	0.293	0.187
<i>1920</i>								
March 20.....	Roots	3.51	0.253	0.300	0.550	0.300	0.277	
April 24.....	Roots	3.69	0.258	0.413	0.541	0.516	0.401	
May 10.....	Tops	45	4.10	0.282	0.512	1.387	1.555	0.679
May 10.....	Roots	55	3.57	0.183	0.425	0.575	0.466	0.362
May 20.....	Tops	48	3.10	0.197	0.472	1.334	1.302	0.502
May 20.....	Roots	52	2.87	0.115	0.387	0.556	0.487	0.377
June 3.....	Tops	61	2.45	0.178	0.423	1.877	1.182	0.473
June 3.....	Roots	39	2.18	0.114	0.432	0.559	0.466	0.393
June 19.....	Tops	80	1.67	0.125	0.391	1.197	0.728	0.432
June 19.....	Roots	20	2.00	0.110	0.383	0.997	0.433	0.428
July 3.....	Tops	86	1.71	0.123	0.343	0.823	0.866	0.461
July 3.....	Roots	14	1.65	0.096	0.302	0.634	0.427	0.420
July 17.....	Tops	82	1.80	0.115	0.310	0.780	0.854	0.402
July 17.....	Roots	18	1.20	0.090	0.305	0.821	0.450	0.420
August 1.....	Tops	78	1.80	0.108	0.285	0.646	0.948	0.373
August 1.....	Roots	22	1.37	0.083	0.297	0.991	0.470	0.419
August 14.....	Tops	70	1.22	0.074	0.267	0.637	0.741	0.270
August 14.....	Roots	30	1.36	0.073	0.293	0.954	0.533	0.417
August 28.....	Tops	73	1.30	0.058	0.200	1.002	0.580	0.233
August 28.....	Roots	27	1.08	0.056	0.288	1.574	0.588	0.349
September 11.....	Tops	76	1.36	0.064	0.199	0.648	0.390	0.198
September 11.....	Roots	24	0.78	0.068	0.203	1.086	0.427	0.353
October 1.....	Tops	79	1.30	0.075	0.129	0.585	0.473	0.218
October 1.....	Roots	21	0.92	0.095	0.193	1.050	0.533	0.316

When the relative amounts of the elements in the roots and tops are compared, the importance of nitrogen and phosphorus for maintaining the plant through the winter is evident.

The importance of sulfur in the plant metabolism is suggested by the large amounts—three to four times greater than the phosphorus—contained in the tops. This will be taken up later in the discussion.

There is not sufficient evidence in these figures to warrant the statement that the roots are absorbing plant-food elements from the soil after the top growth has ceased, but this is a possible explanation of some of the variations in root composition.

#### RELATION OF ROOT AND TOP COMPOSITION TO FALL PLOWING, GROWTH OF FIRST SEASON

Fall plowing is almost a necessity in some sections and is desirable on many farms.

The composition of the roots of sweet clover of the first year is less in percentage and in total amount of plant-food than in the spring of the second year. The weight of organic matter is much less the first year. It is thus evident that soils needing enrichment in nitrogen and organic matter will be helped more by spring plowing than by fall plowing of biennial white sweet clover. The need for improvement of the surface and subsurface should determine the time of fall plowing, if a crop is not to be seeded in the fall after plowing. Plowing early in the fall incorporates in the soil, young tender tops and roots, both of which proceed to decompose rapidly. Such early plowing results in conditions which will cause relatively large losses during the fall and spring. A much larger part of the crop is incorporated in the surface soil when plowed early. Early fall plowing is more satisfactory for killing the crop than late fall plowing. If a crop is to follow immediately, early fall plowing would be justifiable, and would result in considerable enrichment of the surface soil if systematically followed, as can be seen by reference to the analyses of the early fall tops.

Late fall plowing would offer a better opportunity for enriching the deeper layers of the soil, because of the roots having developed more fully and being richer than at earlier stages. The surface is also improved at the same time, as it must be remembered that at all times the largest part of the roots and tops will be plowed into the surface 7 or 8 inches. The improving of the sub-soil will be a much slower process than the improving of the surface or subsurface. Both are equally important, and both should be enriched at the same time, if possible, although the slower rate of enriching the lower layer means that it must receive consideration for a longer time. At any given period the weight of roots below 14 inches is a small proportion of the total root weight. The root development at deeper layers depends upon the acidity and permeability of the soil type. An increasing proportional root development may occur at lower layers through repeated growth of deep-rooted crops that are plowed at a time when rich, plump roots are present in those layers. This will insure leaving the elements of fertility at the deeper layers and in the most decomposable forms, regardless of where they originated.

The rate of nitrification of the late fall growth of sweet clover is less than of the early growth, especially of the tops. This would help check the losses from the practice. Fall plowing, as commonly practiced, is not successful in killing the crop. Cutting a hay crop before translocation, and following it with plowing before growth proceeds much, should aid in killing the fall growth. This effect will depend, however, upon weather conditions prevailing thereafter, and upon how much the roots may have stored before cutting occurred. Cutting the hay crop early and delaying plowing enables the crop to recover its loss of food, and killing is not then effectively accomplished. The vigor of this biennial weed has not yet been reduced by cultivation, and its survival under even adverse conditions is common. The composition of fall cut hay may be judged from the analyses.

TABLE 6  
*Composition of sweet clover, leaves, stems, roots and seeds*  
Pounds per ton on water-free basis

DATE	PART OF PLANT	PROPORTION OF WHOLE PLANT	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1919</i>								
October 15.....	Leaves	16	68.40	3.61	15.84	22.40	57.68	10.74
October 15.....	Stems	24	27.00	1.44	4.24	18.20	14.68	2.18
October 15.....	Roots	60	61.20	4.04	4.82	12.80	6.54	4.84
<i>1920</i>								
July 3.....	Leaves	20	70.80	4.00	15.66	22.40	44.06	10.04
July 3.....	Stems	66	22.80	1.99	3.98	14.80	8.94	1.84
July 3.....	Roots	14	32.00	1.92	6.04	12.60	8.54	8.40
August 28.....	Seeds		99.00	8.99	9.46	16.00	21.62	2.96
September 11.....	Seeds		94.40	8.39	8.80	14.00	22.42	2.78

#### DISTRIBUTION OF THE ELEMENTS IN THE FIRST SEASON'S GROWTH

Nitrogen is contained in both the tops and roots in larger amount than the other elements reported. In a sample of the root nitrogen studied, 86 per cent was water-soluble after grinding. In the stem and leaves only about one-third of that present is soluble. The high solubility of nitrogen in the root accounts for the rapid rate of decomposition of the roots in spite of their woody appearance. This plant stores its nitrogen in a soluble form that is easily moved even under cool growing conditions. This nitrogen is known to be largely organic and is apparently composed of the simpler building stones of legume proteins.

Calcium was found to occupy first place among the mineral elements in the fall tops and second place among them in the fall roots. In fact, in the whole fall plant it exceeds potassium slightly, being second to nitrogen. This high

calcium content is in accord with its large dependence upon the presence of limestone in the soil. There is, however, another significant fact shown by the analyses of the leaves in table 6. Calcium is present in the leaves in very large amounts. Its presence in such amounts in the plant's laboratory suggests it has, among others, a special function in this plant in the manufacture of the nitrogen compounds. It apparently becomes fixed in rather stable forms, otherwise it would be moved about and lost by leaching. It even considerably surpasses potassium in the sweet clover seed. Ames and Boltz (1) reported that the calcium of alfalfa was the least soluble of the three mineral elements, being less than half as soluble as potassium and magnesium, and that it existed in some organic form other than as the oxalate.

Potassium is the third element in quantity in the tops, and second in the roots. The response of sweet clover to potassium feeding is shown by its high content of that element where it has been applied.

Magnesium is present in the tops in much smaller amounts than calcium and potassium. The magnesium content of the seed is very low, which coincides with similar analyses of alfalfa seed, but is directly opposite to that of common clovers. The magnesium content of the leaves of the fall growth greatly exceeds that of the seed, which in turn exceeds that of the stems.

Sulfur is present in the tops in larger amounts in most cases, and in the roots in lesser amounts than the phosphorus. Sweet clover is a sulfur feeder, as judged by these results. It means that large amounts may be removed in the hay. Its high content of sulfur may be attributed to its preventing this element from leaching out of the soil by absorbing it during periods when it would otherwise be lost. At the Spring Valley Experiment Field some sulfur is obtained from the fumes of the heating plant adjacent to it. Three top and root samples taken for the authors by Snider and Hein of the soil experiment field staff on September 11, 20, and 27, 1922, showed 13.72, 14.00 and 14.40 pounds, respectively, of sulfur per ton of tops; and 9.40, 7.20, and 8.20 pounds respectively for the corresponding roots. Whether these large quantities are in excess of the optimum requirements is not known. Whatever the case, this high content is important in considering the sulfur cycle.

On twelve plots studied Ames and Boltz found the average sulfur content of alfalfa hay of first and second cutting higher than that of phosphorus. In alfalfa the sulfur was only 50 per cent soluble, which suggests its close relation to the nitrogen metabolism in such legumes.

Phosphorus is contained in sweet clover in the smallest amount of all the important elements. This deserves special consideration in using the crop for soil enrichment and will be treated under another heading.

#### COMPOSITION OF TOPS AND ROOTS OF GROWTH OF SECOND SEASON

The roots in the spring of the second year appeared to increase in all the elements, except potassium, before a top growth developed. This is shown in table 5. There is not sufficient data to decide whether the increase is real or

apparent. An extension of the root system in cold weather, and an early functioning of new and old nodules probably accounts for the increases noted. The tops of April 24 were about two inches high. Between April 24 and May 10, rapid changes occurred because of a rapid growth of tops.

A gradual decrease in percentage or pounds per ton is seen in the tops for nitrogen, phosphorus, sulfur, calcium, and magnesium, and a general decrease, with two exceptions, for potassium. These decreases were accompanied by large increases in dry matter, as the plants approached maturity.

In the roots nitrogen, phosphorus, and sulfur decrease in an orderly manner, as in the tops. This is shown in table 4. The calcium and magnesium tend to change very little, compared with the other elements. Potassium is again the exception, being very high on several dates and varying from 11 to 31 pounds. In fact, it tends to increase instead of decrease. The reasons for the large potassium content of the tops on June 3 and August 28 and in the roots on June 19 and August 28, are not known, but the authors suggest that this element may be needed in large amounts at special periods in the plant's development, such as preceding flowering and during seed ripening. These large amounts may represent a tolerance, but this seems unlikely, as the other elements are not present in large amounts on those dates.

#### RELATION OF ROOT AND TOP COMPOSITION TO SPRING PLOWING, GROWTH OF SECOND YEAR

Nitrogen is the most important element in the sweet clover from the standpoint of its use in improving soils and supplying nitrate rapidly for the succeeding crop. The amount of nitrogen in the whole plant in the spring depends upon the weather conditions that prevail. In some seasons the crop was found to contain about 200 pounds of nitrogen per acre in the tops alone by May 10. In other seasons the tops contained about 75 pounds, because of a growth retarded by the season being later. Soils needing enrichment in nitrogen and organic matter should have as much of the second year growth plowed under as possible without danger of injury to the succeeding crop.

After the crop has been successful on the same field two or three times, it may be plowed under earlier, because of less need for the largest amount of active organic matter.

The enrichment of the subsurface and subsoil will be accomplished more rapidly if the crop is plowed after the roots have started to increase in mineral elements and nitrogen in the spring, but before much material has moved into the tops from the roots. The results show this date to be about May 10 for the conditions thus far studied.

If no crop is to follow the sweet clover, it may well be left to complete its full growth and to add all the material to the soil. When so left to mature, the rate of nitrification is very slow if the mature dried material is plowed under in the fall. This is a decided advantage, as less nitrate is lost under these conditions.

## COMPOSITION OF LEAVES, STEMS, ROOTS, AND SEEDS

The composition of the leaves, stems, and roots, in fall and summer samples, and also the analysis of samples of seeds are included in table 6.

The richness of the leaves and the importance of conserving them is apparent from the data. This is of importance in connection with the use of the crop for hay. The high content of the stems in potassium and nitrogen is likewise apparent. The very high calcium content of the leaves suggests an important

TABLE 7  
*Composition of sweet clover roots at 0-7 and 7-40 inches*  
Pounds per ton on water-free basis

ELEMENT	SAMPLE 1		SAMPLE 2	
	Root depth in inches			
	0-7	7-40	0-7	7-40
Nitrogen.....	64.80	67.00	67.20	64.00
Phosphorus.....	5.28	5.12	5.78	4.64
Sulfur.....	5.32	5.00	5.78	5.26
Potassium.....	13.34	11.60	12.00	12.36
Calcium.....	7.20	7.60	6.40	7.20
Magnesium.....	5.32	5.44	4.48	5.12

TABLE 8  
*Composition of sweet clover tops (second year's growth) as influenced by soil treatment*

	POUNDS PER TON WATER-FREE BASIS	INCREASE FOR TREATMENT	
		pounds	per cent
Calcium content on limed land (Urbana).....	40.00		
Calcium content on unlimed land (Urbana).....	31.00	9.00	29.0
Phosphorus content on phosphated land (Exp. fields)....	8.34		
Phosphorus content on unphosphated land (Urbana)....	5.64	2.70	48.9
Potassium content on potassium treated land (Urbana)...	47.40		
Potassium content on non-potassium treated land (Urbana).....	28.60	18.80	65.7

function of this element in the metabolism of sweet clover and has been already discussed. The leaves of alfalfa were reported by Ames and Boltz as containing 30.4 pounds of calcium on the basis of the figures in table 6. Our results show 57.6 and 44 pounds respectively for fall and summer samples. Calcium is present in the seed in very high amounts. This is unusual for this element, but is in accord with its exceptionally high content in the leaves, and it may bear a relation to some special function not yet studied in sweet clover.

Magnesium is present in the smallest amount in the seed and is practically the same, as the above authors reported for alfalfa seed.

Sulfur is contained in the leaves, seed, stems, and roots in larger amounts than phosphorus. Sulfur may be tolerated in the leaves, but it could hardly be expected to be tolerated to any large extent in the seed. It seems that sulfur, as well as calcium, needs more study from the new standpoint of its relation to the plant metabolism.

#### COMPOSITION OF ROOTS AT DIFFERENT DEPTHS

In table 7 analyses of root samples taken in the fall at 0 to 7 and 7 to 40 inches are included. The root composition in the two layers does not differ beyond the error of sampling. This is evidence that the roots at considerable depth maintain the same percentage composition as they do near the surface.

#### INFLUENCE OF SOIL TREATMENT ON COMPOSITION OF SWEET CLOVER

In the course of various investigations, data showing the influence of soil treatment on the composition of sweet clover tops have been obtained. In table 8, some of these results are presented.

In the calcium comparison, both samples came from brown silt loam, one treated with limestone and the other untreated. Another analysis made, but not included in table 8, was a composite from six experiment fields and shows 37.14 pounds of calcium per ton, where five of the six fields are located on what was originally very poor soil. The samples from phosphated land came from soil which was untreated with potassium and included a composite of six experiment fields, whereas the unphosphated sample grew on soil naturally richer in all elements than five of the six fields from which the composite was made. The potassium comparison samples came from brown silt loam fields, located near each other. The large intake of potassium, where it was applied, is important, and may account in part for the beneficial effects sweet clover produces on other crops when it is plowed under. On a number of the Illinois experiment fields where potassium is applied the sweet clover is decidedly improved. The increases in the corn crop produced by sweet clover on the potassium plots are traceable to the influence of the potassium, perhaps as much as to the nitrogen of the sweet clover. The potassium applied is certainly not lost in the open soils to the deep-rooting sweet clover. The calcium, potassium, and phosphorus content of sweet clover roots may be influenced by soil treatment in the same manner as other crops. The sulfur analyses from Spring Valley may be considered as representing a sulfur-treated sample, and may be compared with those in the other tables. When so compared, here also an influence is evident from the treatment.

It is doubtful if any other factor is important when compared with that of soil treatment for this hardy crop. Inoculation is understood to be in the nature of treatment from a fertility standpoint. The nitrogen content of inoculated sweet clover has been found to be very high from many different soils.

SWEET CLOVER COMPOSITION COMPARED WITH CORN AND WHEAT PLANT-FOOD REQUIREMENTS

The plant-food contained in sweet clover tops and roots, at the time it would be plowed as a green manure, is calculated on an acre basis from known ratios of roots to tops, and from known weights per acre. These are shown in table 9 and are taken from the May 10 sample of table 5. On untreated normal corn belt soil, sweet clover is deficient in phosphorus, and in potassium, as measured by crop requirements. The large response to these elements where rock phosphate and potassium salts have been applied supports the view that the success of sweet clover may be retarded by a deficiency of these elements in many soils. If the analysis of the samples from the experiment fields, where rock phosphate has been applied, and from the North Farm at Urbana, where potassium has been applied, are measured by crop requirements, then sweet clover would carry sufficient potassium for a corn crop with 20 pounds to

TABLE 9  
*Sweet clover composition compared with wheat and corn plant-food requirements*

ELEMENT	2600 POUNDS TOPS	3100 POUNDS ROOTS	TOTAL POUNDS	PLANT-FOOD REQUIRED BY	
				100 bushels corn	50 bushels wheat
Nitrogen.....	108.7	110.7	219.4	150.0	96.0
Phosphorus.....	7.3	5.7	13.0	23.0	16.0
Sulfur.....	13.3	13.2	26.5	15.3	11.8
Potassium.....	36.0	17.8	53.8	71.0	58.0
Calcium.....	40.4	14.4	54.8	22.0	11.0
Magnesium.....	12.3	12.2	24.5	17.0	8.0

spare, but would still fall short in phosphorus about 4 pounds for the corn, although exceeding the requirements for wheat.

Although it is true that the crops of sweet clover being produced are the basis of the figures used, and the corn crops raised are much lower than the standards employed here; such a relation is no excuse for applying actual average yields, as, in many cases where sweet clover is being used, 100 bushels of corn and 50 bushels of wheat are not too high to expect, and these figures may need to be raised for future study with improved conditions of production.

Possibly 85 to 90 per cent of the fertility elements in the sweet clover becomes available to the succeeding crops. In such a case the margin of safety of available phosphorus would not be large even with soil treatment. The figures emphasize the need for phosphate applications before seeding the legume and when it is plowed under. Potassium may be applied with best results prior to seeding.

Attention to meeting the fertility demands of legumes used in soil improvement must not be overlooked, otherwise production of succeeding crops may be limited through deficiencies existing in the green manure.

Sweet clover, although a good ravager, is also a good feeder, responding well when fed. These two characteristics open the way for attaining the highest composition in the important elements for either soil improvement or feed. Where a rapid improvement of soil is sought, limestone, phosphorus, and potassium should be applied on many soils for this crop.

#### SUMMARY

The composition of sweet clover tops and roots has been determined at various periods in its entire growth. The tops decrease in nitrogen, phosphorus, and sulfur with maturity the first season. The roots increase in nitrogen and apparently in other elements while increasing in dry matter. In the second season the roots increase slightly at first and then decrease somewhat parallel to the decrease in the tops which occurs at the same time. These changes are regarded as due in part to translocation, although the evidence is not sufficient for final proof.

Nitrogen is the outstanding element in the composition of both the tops and roots at most periods when the samples were taken. A high percentage of the total nitrogen is found in a soluble form in the roots.

The mineral elements are present in quantity, generally in the following order: potassium, calcium, magnesium, sulfur, and phosphorus.

Potassium appears to be required in relatively large amounts by this crop. A large response to potassium feeding has been noted. A high content, suggesting a large requirement for some special metabolic processes, was found at several periods.

Calcium is also demanded in large amounts for sweet clover. All its functions are not known, but it would appear that it is either needed in larger amounts for its known functions or that it fills a special and as yet unrecognized place in the metabolism of sweet clover. It is present both in the seed and in the leaves in excess of other elements except nitrogen.

Magnesium is present in normal amounts in the seed, being about one-third that of the phosphorus content. It is very high in the leaves, being two to three times that of the phosphorus content.

Sulfur is present in sweet clover in large amounts from a relative viewpoint. Out of 47 comparisons with phosphorus it exceeds that element in 42 cases. It is high even in the seed, and very high in the leaves. It is not nearly so high in the seed in proportion to phosphorus as it is in the leaves.

Phosphorus was found in the smallest amounts of all elements studied. It was higher in the fall roots than in other samples. Applying this element for the use of the growing sweet clover and again when it is plowed under offers an excellent opportunity to make it more available for succeeding crops.

The composition of the roots is nearly constant at the depths studied.

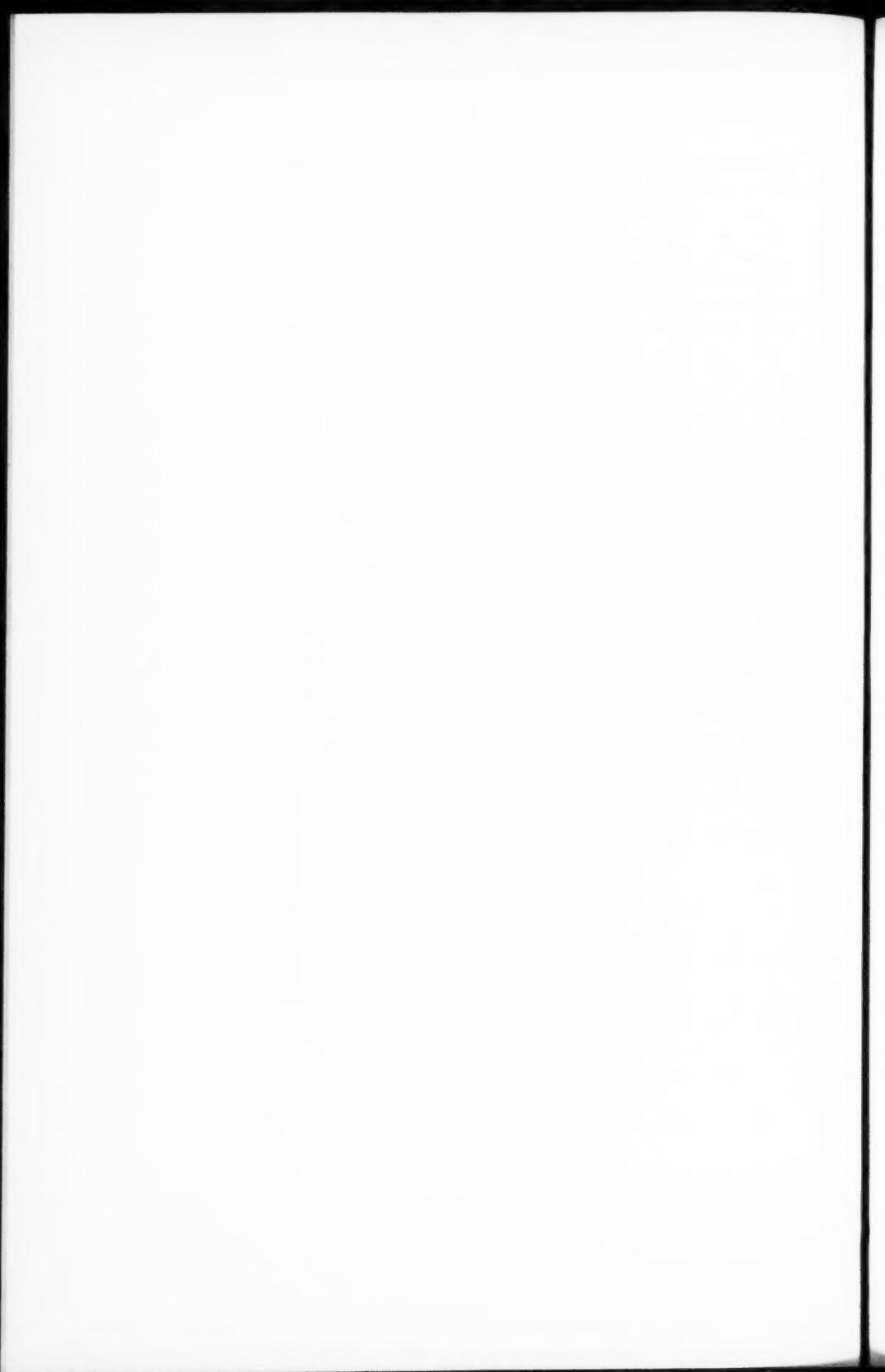
The composition of sweet clover may be considerably influenced by applications of limestone, phosphorus, and potassium.

Fall plowing, although adding much organic matter and nitrogen, will not add as much material as spring plowing, if the crop has made as much growth as possible before the usual time of plowing for corn.

The extensive root system existing in late fall and early spring affords an opportunity, if plowed at these periods, to enrich deeper layers than the surface soil. Either fall or spring plowing of this crop will be highly beneficial because of its large tonnage per acre and its relatively high content of nitrogen, potassium, calcium, and sulfur.

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## A STUDY OF PHYSIOLOGICAL BALANCE FOR ALFALFA IN SOLUTION CULTURES<sup>1</sup>

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Physiological balance investigations on plants have been carried on for several years by various workers. The solution culture method lends itself very conveniently to this kind of study, especially since this method has become systematised through the work of Tottingham (7) and of Shive (6). Studies of the ratios of the elements known to be of importance in the plant economy, have by no means lost in interest. While Loew's (2) theory of the calcium-magnesium ratio is still being contested in some quarters, other students of the question are convinced that ratio relationships play a vital part in plant nutrition. Lemmermann and Einecke (1) for instance, conclude on the basis of their experiments, that there must exist optimal ratios not only between calcium and magnesium, but between the other nutrients as well.

Cereals and soybeans stand out prominently among the plants experimented with in physiological balance studies. Alfalfa, properly handled, can be grown in solution cultures for a considerable length of time—the writer (3) has grown one series of plants in solution cultures for 98 days—it can also possibly be made to yield more than one crop, and thus afford the benefit of a more extended consecutive period of observation and study. If, in addition, the great economic importance of this legume is considered, the incentive offered by it for this kind of study is obvious.

### METHOD OF PROCEDURE

The alfalfa seed used in the present work came from a lot which furnished the seeds for some previous studies with alfalfa in solution cultures (3, 4) and in soils. The technique for germination, transfer to net and to jars, renewal of solutions, measurements of water absorption and supply of iron, harvesting, drying, and analysis, was the same as described in connection with those studies.

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<sup>2</sup> Thanks are due Dr. John W. Shive, of the department of plant physiology, for his continuously encouraging attitude and for his valuable suggestions in connection with the present work.

Twenty-one solutions in duplicate, in jars with 3 plants to a jar, were used in the present series, and the culture solution R<sub>5</sub>C<sub>2</sub> (Shive, 1.75 atm.), in triplicate, served as a check. The seedlings were transferred from the germination dish to the seedling net on August 20, 1923, two days after sowing. On August 29, the plantlets were transferred to the jars in which they were grown until November 14, 1923.

The type of solutions used in this experiment is that designated as type "I" in a plan for co-operative research on the salt requirements of representative agricultural plants prepared for a special committee of the division of biology and agriculture of the National Research Council (5). All the solutions had a

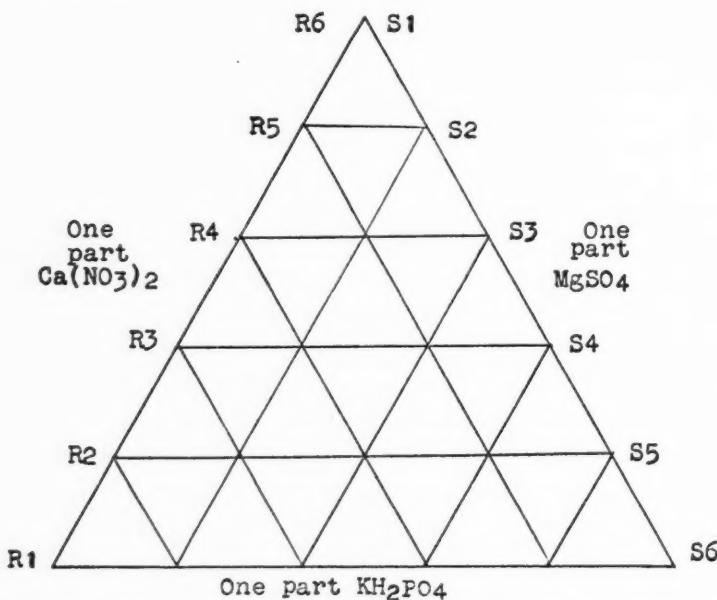


FIG. 1. DIAGRAM SHOWING SOLUTION NUMBERS AND VOLUME-MOLECULAR PROPORTIONS OF THE THREE SALTS

total concentration of one atmosphere possible osmotic pressure. These 21 solutions, as explained in the publication referred to, are arranged in such a manner as to give all possible combinations, in variations of one-eighth of the total volume-molecular concentration of the three salts—monopotassium phosphate, calcium nitrate, and magnesium sulfate—which make up the solutions. A triangular diagram as shown in figure 1, with lines running parallel to the three sides, respectively, is laid off so that each point of intersection represents a different solution. These points, beginning with the one at the lower left hand (R<sub>1</sub>S<sub>1</sub>), and running to the right in the row, with the rows running from

the base to the apex of the triangle, are designated by the solution numbers in the order arranged in table 1.

The partial volume-molecular concentrations and the molecular proportions of the solutions used, as well as those of the check solution, are given in table 1.

TABLE 1

*Partial volume-molecular concentrations and molecular proportions of  $KH_2PO_4$ ,  $Ca(NO_3)_2$ , and  $MgSO_4$ , used in the culture solutions*

Total osmotic value, approximately 1.00 atmosphere, except in the check, which is 1.75 atmospheres.

SOLUTION NUMBER	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			MOLECULAR PROPORTIONS		
	$KH_2PO_4$	$Ca(NO_3)_2$	$MgSO_4$	$KH_2PO_4$	$Ca(NO_3)_2$	$MgSO_4$
R <sub>b</sub> C <sub>2</sub> , check	0.0180	0.0052	0.0150	3.77	1.09	3.14
IR <sub>1</sub> S <sub>1</sub>	0.0027	0.0027	0.0161	1	1	6
S <sub>2</sub>	0.0025	0.0049	0.0123	1	2	5
S <sub>3</sub>	0.0024	0.0071	0.0094	1	3	4
S <sub>4</sub>	0.0022	0.0089	0.0067	1	4	3
S <sub>5</sub>	0.0022	0.0108	0.0043	1	5	2
S <sub>6</sub>	0.0020	0.0122	0.0020	1	6	1
R <sub>2</sub> S <sub>1</sub>	0.0053	0.0027	0.0132	2	1	5
S <sub>2</sub>	0.0049	0.0049	0.0099	2	2	4
S <sub>3</sub>	0.0047	0.0071	0.0071	2	3	3
S <sub>4</sub>	0.0045	0.0090	0.0045	2	4	2
S <sub>5</sub>	0.0041	0.0104	0.0021	2	5	1
R <sub>3</sub> S <sub>1</sub>	0.0076	0.0025	0.0101	3	1	4
S <sub>2</sub>	0.0072	0.0048	0.0072	3	2	3
S <sub>3</sub>	0.0068	0.0068	0.0045	3	3	2
S <sub>4</sub>	0.0065	0.0086	0.0021	3	4	1
R <sub>4</sub> S <sub>1</sub>	0.0099	0.0025	0.0074	4	1	3
S <sub>2</sub>	0.0094	0.0047	0.0047	4	2	2
S <sub>3</sub>	0.0090	0.0068	0.0022	4	3	1
R <sub>5</sub> S <sub>1</sub>	0.0123	0.0024	0.0049	5	1	2
S <sub>2</sub>	0.0118	0.0047	0.0023	5	2	1
R <sub>6</sub> S <sub>1</sub>	0.0145	0.0024	0.0024	6	1	1

## DISCUSSION OF RESULTS

The relative values for dry weights, water absorption, water requirement, and nitrogen content, separately for tops, roots, and whole plants, as compared with the check taken as 100.0, and the actual figures for the check, are given in table 2. The actual values for all these data are presented graphically in figure 5, the cultures in this presentation being arranged in order of the highest

TABLE 2  
*Relative dry weights, water absorption, water requirements, and nitrogen content of tops, roots and whole plants, of alfalfa grown 77 days in solution cultures of type "I," from August 29 to November 14, 1923*

Figures in parenthesis are actual values

SOLUTION NUMBER	YIELD			WATER REQUIREMENTS			NITROGEN, PER CENT			NITROGEN, GRAMS		
	Tops	Roots	Whole plants	WATER ABSORPTION	Tops	Roots	Whole plants	Tops	Roots	Whole plants	Tops	Roots
R <sub>5</sub> C <sub>2</sub> check	100.0 (3.895)	100.0 (1.352)	100.0 (5.247)	100.0 (2,237)	100.0 (576.6)	100.0 (1,652.6)	100.0 (426.6)	100.0 (3.38)	100.0 (3.03)	100.0 (3.29)	100.0 (0.1317)	100.0 (0.0410)
IR <sub>5</sub> S <sub>1</sub>	103.0	90.6	99.8	108.7	104.8	120.8	108.6	110.7	85.5	105.5	113.9	77.0
S <sub>2</sub>	105.8	84.6	100.4	115.2	108.0	130.8	114.2	115.3	89.1	119.6	122.1	75.3
S <sub>3</sub>	116.8	102.5	113.1	114.3	97.1	112.1	97.8	111.5	76.9	104.2	130.2	78.8
S <sub>4</sub>	112.5	89.3	106.5	115.8	101.9	129.3	108.0	109.1	85.1	104.8	122.7	76.1
S <sub>5</sub>	132.4	97.6	123.6	132.6	100.0	141.1	108.1	111.8	85.8	107.6	148.0	83.6
S <sub>6</sub>	108.2	76.5	100.0	102.3	93.7	135.0	101.8	113.0	90.4	109.7	122.3	68.8
R <sub>5</sub> S <sub>1</sub>	83.8	69.3	80.0	89.0	105.6	127.9	110.7	113.6	84.4	107.2	94.3	58.5
S <sub>2</sub>	101.6	97.7	100.6	107.0	104.8	112.8	105.9	105.9	92.0	102.7	107.6	90.0
S <sub>3</sub>	119.1	108.5	116.4	115.4	97.1	105.0	99.3	110.3	107.2	131.4	104.1	124.9
S <sub>4</sub>	134.9	103.1	126.7	137.9	101.3	133.7	108.2	114.5	80.5	108.5	154.5	82.9
S <sub>5</sub>	94.7	82.0	91.4	93.2	98.4	113.2	101.8	107.9	85.5	103.3	102.2	70.0
R <sub>5</sub> S <sub>1</sub>	99.1	82.6	94.9	96.1	97.3	116.1	101.0	108.8	94.0	106.3	107.8	77.5
S <sub>2</sub>	122.6	92.7	114.9	124.2	99.8	132.5	106.8	109.1	97.3	107.3	133.9	90.2
S <sub>3</sub>	97.8	89.4	95.6	107.9	107.3	117.1	109.9	104.7	80.1	99.3	102.4	71.7
S <sub>4</sub>	89.4	75.5	85.8	96.4	106.9	122.7	112.0	108.5	86.1	104.2	97.0	64.9
R <sub>5</sub> S <sub>1</sub>	109.2	85.3	103.0	111.9	101.4	130.8	107.8	112.4	96.7	109.7	122.7	82.4
S <sub>2</sub>	99.8	78.2	94.2	121.1	121.3	154.1	128.7	103.8	91.0	101.5	103.6	71.2
S <sub>3</sub>	81.5	85.2	82.4	84.3	99.8	99.2	99.4	102.9	94.0	100.6	83.9	80.2
R <sub>5</sub> S <sub>1</sub>	87.6	61.7	80.9	84.6	96.3	141.1	104.9	111.8	89.7	108.5	97.9	55.3
S <sub>2</sub>	107.4	91.2	103.2	99.7	92.1	112.5	95.9	102.3	86.1	99.3	109.9	78.5
R <sub>5</sub> S <sub>1</sub>	100.9	72.8	93.7	96.6	95.6	133.2	103.1	107.9	107.2	108.5	109.0	78.0

yields of tops. The areas representing the highest 7 yields, separately for tops, roots, and whole plants, are outlined on the triangular diagrams (figs. 2, 3, and 4).

### *Yields*

It will be noted that the points on the triangle (fig. 2) representing the solutions producing the highest 7 yields of tops, are comprised within one continuous area, by far the greater part of which, covering 5 out of the 7 solutions, lies in the region characterized by a rather high molecular proportion of calcium nitrate. The lowest of this group of high top yields—solution R<sub>4</sub>S<sub>1</sub>—has the

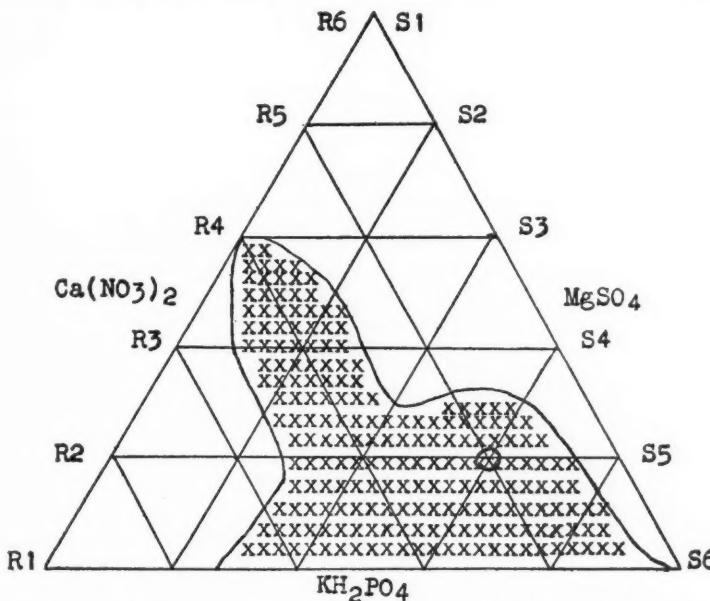


FIG. 2. DIAGRAM SHOWING POSITION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF TOPS

lowest molecular proportion of calcium nitrate in the group. The two solutions R<sub>2</sub>S<sub>4</sub> and R<sub>1</sub>S<sub>5</sub>, producing respectively the highest and the next highest top yield of this group, have volume-molecular proportions of 2-4-2 and 1-5-2, respectively, for monopotassium phosphate, calcium nitrate and magnesium sulfate. As seen from table 2, compared with the top yield of the check, R<sub>5</sub>C<sub>2</sub> (Shive, 1.75 atm.), taken as 100.0, the values for solutions R<sub>2</sub>S<sub>4</sub> and R<sub>1</sub>S<sub>5</sub>, are 134.9 and 132.4, respectively.

When the values for root yields are considered, it will be noticed, that the point representing the solution, R<sub>2</sub>S<sub>3</sub>, which produced the highest yield, is shifted on the triangle, in the same row, one place to the left of that representing

the solution,  $R_2S_4$ , giving the highest top yield. The volume-molecular proportions of  $R_2S_3$  are 2-3-3 for monopotassium phosphate, calcium nitrate, and magnesium sulfate, respectively, as against 2-4-2 for solution  $R_2S_4$ , which produced the highest top yield. Solution  $R_5S_2$ , with molecular proportions of 5-2-1 for the salts indicated, is included in the group of the seven solutions producing the highest root yield. This solution, which is high in monopotassium phosphate and low in magnesium sulfate, produced one of the highest top yields in the group of seven which gave medium yields of tops.

The configuration of the area on the triangle, representing the region of

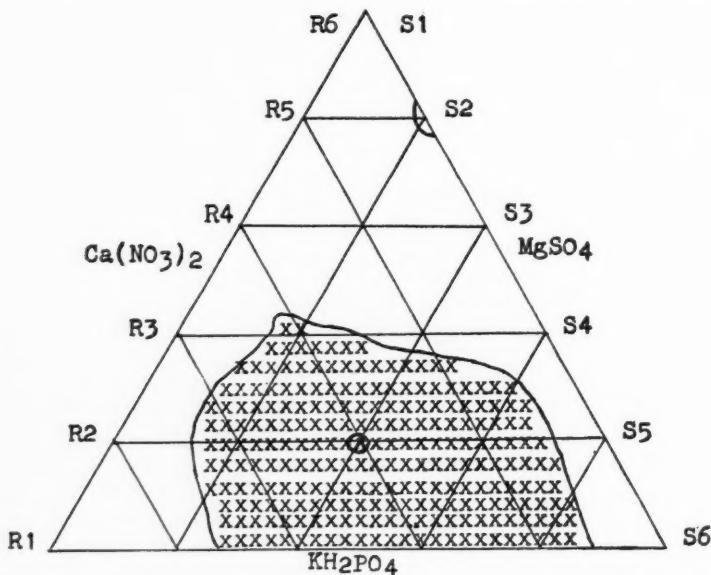


FIG. 3. DIAGRAM SHOWING POSITION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF ROOTS

The highest yield in the group is marked with a circle

highest yield of whole plants is in general the same as that representing the region of highest top yield, except that solution  $R_4S_1$ , with molecular proportions of 4-1-3, is left out and its place taken by solution  $R_5S_2$ , with molecular proportions of 5-2-1; this last solution, as already stated, produced one of the highest medium yields of tops, and also gave a high root yield. The point representing the solution producing the highest yield for the whole plant, is the same as that representing the solution producing the highest yield for tops.

From figure 5, it is seen that the short line to the left, representing the top yield of the check solution,  $R_5C_2$  (Shive, 1.75 atm.), falls below all but 8 of the 21 solutions of type "I," represented on the curve for top yields. Of these 8,

the values for 2 are substantially the same as that for the check. On the other hand, all the points but 3, on the curve representing the root yield of those 21 solutions, fall below the line that corresponds to the check. In the curve representing the whole plant yield of the 21 solutions, 9 fall below the line of the check. In general, on account of the relatively higher root yield of the check, the divergence in the yield values of tops in favor of the solutions of type "I" as compared with the check, becomes somewhat lessened when the corresponding values for the whole plants are compared. There is some difference in the average ratios of root yield to that of whole plant, between the solutions of

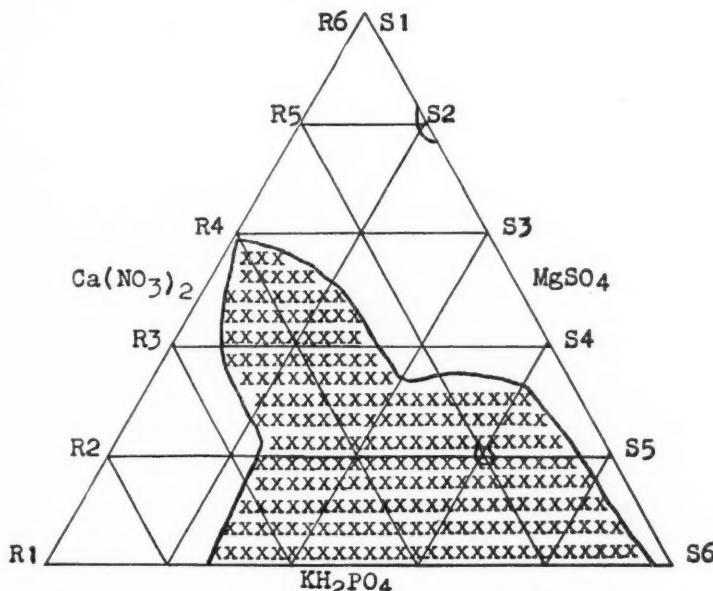


FIG. 4. DIAGRAM SHOWING POSITION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF WHOLE PLANTS

The highest yield in the group is marked with a circle

type "I" and the check solution. The average value of this ratio for the former is 0.22, for the latter it is 0.26; or, expressed on the basis of the check ratio taken as 100, that of the solutions of type "I" will have the value of 85.

It should be mentioned that a series of cultures like the one here discussed had been conducted previously as a preliminary experiment during a short period of growth, but no detailed study was made of the results. It was noted, however, that the highest yield of tops, of roots, and consequently also of whole plants, was produced by solution R<sub>2</sub>S<sub>4</sub>, the same solution that gave the highest yield in the present series.

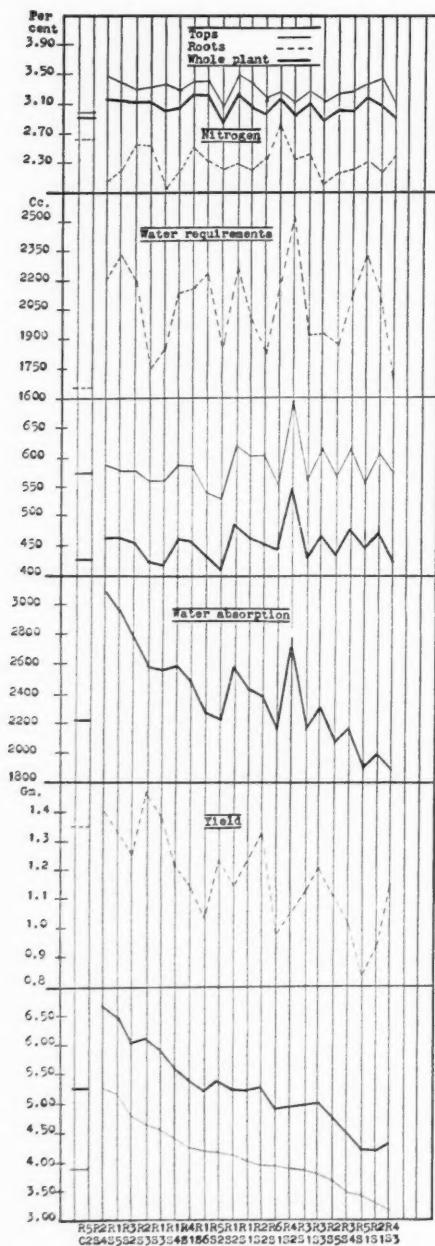


FIG. 5. GRAPHIC SUMMARY OF DATA DETERMINED  
Checks are indicated by short horizontal lines to left

*Water absorption and water requirements*

Except for 8 solutions, which produced a lower total yield than the check, the series of solutions of type "I" showed a higher value for water absorbed than the check. Within the series itself, there is noted a tendency for the curve of water absorption to follow, in a general way, the curve of top yield. An unusually high absorption of water occurred in case of solution  $R_4S_2$ , with a molecular proportion of 4-2-2, for the three salts in the order usually given.

In studying the values for water requirements (cubic centimeter liquid absorbed per gram of dry matter produced), it is noticeable, that from the point of view of top production, there is a tendency for greater efficiency in the utilization of the water absorbed, as the yield goes up higher. The average relative water requirement value for tops for the group of 9 solutions with relative top yield above 107, is 98.2, whereas the average relative water requirement value for the other 12 solutions, goes up to 103.8.

It will be noted further, that although there is in general no great variation in the water requirement values for tops between the check solution and the others, a decided difference is seen to exist when the water requirements of the roots are considered. Of the 21 solutions of type "I," 20 show water requirement values for roots ranging between 105 and 154 as compared with 100 for the check. This high water requirement for roots of the solutions of type "I," naturally brought the average water requirement values of those solutions for whole plants above that of the check. All this is related to the fact mentioned, that the check solution, whether on account of its higher concentration, or because of the particular distribution of the proportions of the tree salts in that solution, is conducive to a relatively greater root development of alfalfa, whereas the solutions of type "I," favor a relatively higher development of tops.

*Nitrogen content*

Notwithstanding the fact that the check solution contained a higher concentration of calcium nitrate than 11 out of the 21 solutions of type "I," the nitrogen percentages of the tops from these latter solutions are invariably higher than that of the tops from the check solution. This appears somewhat remarkable if it is kept in mind that the solutions of type "I" produced on the average higher top yields than the check solution. Precisely the reverse relationship is observed with respect to the nitrogen content of the roots. In this case, all solutions but one of type "I," produced roots with a lower nitrogen percentage than did the check solution. The check solution, it should be remembered, produced at the same time a higher average root yield than that produced by the other solutions. The nitrogen content of tops and of roots in the present case was apparently not governed by the usually accepted principle that a high percentage may be expected to go with a relatively high supply of that element in the medium, or with a low crop yield.

Because of the relatively greater proportion of tops to roots in the whole plant, and to the higher percentage of nitrogen in the tops, the values for the nitrogen percentage in the whole plants of the solutions of type "I," continue on the average to lie above that of the whole plants of the check solution.

The results obtained with the solutions of type "I" in this investigation, with respect to the growth of alfalfa during the first 77 days, may be summarized as follows:

1. The highest yield for tops as well as for whole plants, was obtained with solution  $R_2S_4$ , with molecular proportions of 2-4-2, and a partial volume-molecular concentration of 0.0045, 0.0090 and 0.0045, for monopotassium phosphate, calcium nitrate and magnesium sulfate, respectively. This solution produced a top yield of 134.9 and whole plant yield of 126.7, as compared with top and whole plant yield, respectively, of the check,  $R_5C_2$  (Shive, 1.75 atm.), taken as 100.0.

2. The highest root yield was obtained with solution  $R_5S_3$ , characterized by molecular proportions of 2-3-3, and partial volume-molecular concentration of 0.0047, 0.0071 and 0.0071, respectively, for the three salts indicated. This solution produced a root yield of 108.5 as compared with the root yield of the check taken as 100.0. The regions on the three triangles representing, respectively, the solutions producing highest yields of tops, roots, and whole plants, differ only a little from one another.

3. The average for top yields is higher for the solutions of type "I" than for that of the check. The reverse holds true with respect to root yield. This would indicate that the solutions of type "I" are conducive to a relatively greater top growth than is the check solution.

4. A tendency was shown in the solutions of type "I" toward a greater economy in the use of water as the yields went higher.

5. There was not much difference in the water requirements for tops, between the solutions of type "I" and that of the check.

6. The water requirement values for roots, of the solutions of type "I," are considerably above that of the check.

7. The nitrogen percentage is higher in the tops from the solutions of type "I," than in those from the check solution.

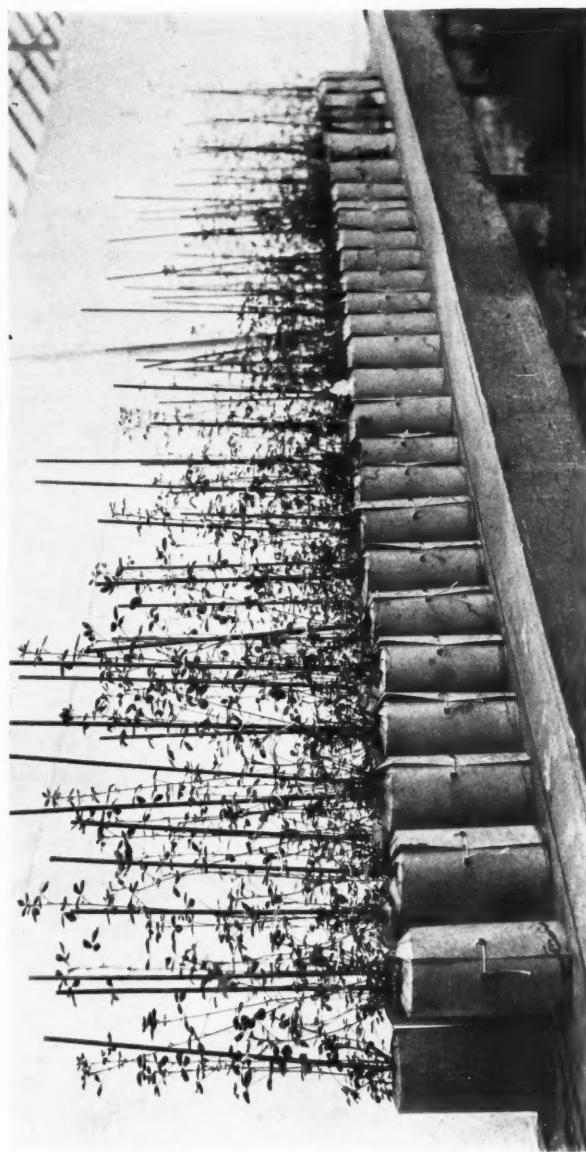
8. The nitrogen percentage is lower in the roots from the solutions of type "I," than in those from the check solutions.

9. The nitrogen percentage of the whole plants from the solutions of type "I," is higher than in those from the check solution.

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S. LOMANITZ



ALFALFA FIVE WEEKS OLD, GROWN IN SOLUTION CULTURES



## FIXATION OF CALCIUM-MAGNESIUM FROM BURNT LIMES, LIMESTONE AND DOLOMITE INCORPORATIONS IN TWO SOIL ZONES

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Frear (3) pointed out that the beneficial effects of limestone particles upon unfavorable acid soil conditions prevail only after the disintegration of the limestone. It is also true that the maintenance of the ameliorated conditions thus brought about is dependent upon the nature of the absorption complexes and upon the tenacity with which absorbed calcium or magnesium is held against hydrolytic disintegration and leaching. It is likewise apparent that the addition of caustic forms of alkali-earths to a soil containing an abundance of hydrated silica will result (11, 12) at first in the formation of at least some normal silicates, compounds which are hydrolyzed much more readily than the more complex and ultimate alumino-silicates. The response of absorbed calcium-magnesium to hydrolysis influences (a) future supplies of plant nutrients, (b) solubility of amphoteric elements, (c) adaptation of the soil as a medium for bacterial activities, and (d) neutralization of the products formed by such activities.

In the absence of plant growth, the measure of the fixation of a definite alkali-earth carbonate addition is the *difference* between the amount added and the amount accounted for jointly by (a) the disintegration of added, or derived, carbonates and by (b) the amount of alkali-earth outgo, during a given time. This paper records the fixation of added alkali-earths extant at the end of four years, as such fixation was influenced by form, fineness, and zone-of-incorporation in outdoor lysimeters. The Ca-Mg fixation results are given in terms of pounds of  $\text{CaCO}_3$  per 2,000,000 pounds of soil, and as per cent of addition, and represent the differences between a constant equivalent of alkali-earths and the several amounts accounted for jointly by carbonate disintegration and by leaching from surface-zone and subsurface-zone incorporations.

### EXPERIMENTAL

The liming materials used were hydrated high-calcic lime, burnt dolomite, a corresponding mixture of separately calcined  $\text{CaO}$  and  $\text{MgO}$ , and four

<sup>1</sup> The results were obtained by means of a fellowship endowment maintained by the National Lime Association and equipment donated by the American Limestone Company. Previous acknowledgment has been made of assistance rendered by sometime Fellows.

separates and separate-composites of limestone and of dolomite, in a constant equivalence of 3570 pounds of  $\text{CaCO}_3$  (2000 pounds of  $\text{CaO}$ ) per 2,000,000 pounds of fallow soil, moisture-free basis. Analysis of soil and alkali-earth additions, make-up of the washed separates, and details of treatment have been given and the lysimeter installation has been illustrated in a previous contribution (13). An 8-inch stratum of surface soil only was used throughout, and each soil charge rested upon a sand filter bed. In one series of tanks the  $\text{Ca}-\text{Mg}$  additions were incorporated throughout the upper half, or zone, and nothing was added to the lower zone. In a parallel series the additions were incorporated only in the lower zone. Before treatment and placement, the soil was thoroughly mixed to insure uniform composition. After incorporation of  $\text{Ca}-\text{Mg}$  materials the two zones were demarcated by means of asphaltum-coated galvanized iron wire discs. The soil was not stirred during the 4-year period of exposure. No samples of soil were taken from either zone until the end of the 4-year period, so that the results show the *ultimate fate* of the incorporations, but not the speed of the processes responsible for their disintegration and depletion.

#### DISCUSSION

As used throughout the text, the expression *fixation* connotes the final increase in the soil's content of non-carbonate  $\text{Ca}-\text{Mg}$  derived from either (a) the initial direct reaction between soil and hydroxides together with the additional reaction between soil and carbonates which came from such hydroxides or (b) the disintegrations of the several limestone and dolomite incorporations. *Fixation* is not used as synonymous with *absorption*, since the absorbed alkali-earths were subject to the diminishing action of leaching, the results of which are designated as *outgo*. The expression *disintegration* is used to designate the difference between added and residual carbonates, and represents the sum of fixation and outgo.

The persistence of carbonates resulting from alkali-earth additions to the upper and lower zones is given as carbonate increases, as compared with controls, in columns 1 and 6, respectively, of table 1. The difference between the constant addition and each carbonate-residual represents the sum of a fixation and concomitant leaching and is given in columns 2 and 7 for the surface-zone and subsurface-zone incorporations, respectively. The total calcium-magnesium outgo from each tank, corrected for the average obtained from that of the controls, is given in columns 3 and 8 for the upper and lower zones of incorporation, respectively. The fate of each incorporation, as accounted for by fixation, leachings, and residual carbonates, is graphed for the surface and subsurface zones. (Figs. 1 and 2.)

Parallel studies on nitrate and sulfate outgo (7) show that those salts are not retained in appreciable quantities by this soil, when exposed to prevailing rainfall, and they justify the assumption that the bases unaccounted for by the combination of residual carbonate and leaching may be designated as fixed,

TABLE I  
Fixation of  $\text{Ca}-\text{Mg}$  from a 3570-pound  $\text{CaCO}_3$ -equivalence (2000 pounds  $\text{CaO}$ ) and of  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , and limestone and dolomite separates in

TABLE 1  
Fixation of Ca-Mg from a 3570-pound  $\text{CaCO}_3$ -equivalence (2000 pounds CaO) and of  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , and limestone and dolomite separates in surface-zone and subsurface-zone incorporations with a loam soil under outdoor conditions for a period of 4 years  
Results are given in terms of  $\text{CaCO}_3$ -equivalence per 2,000,000 pounds of soil, moisture-free basis

TREATMENT	SUBSURFACE-ZONE INCORPORATION						FIXATION FROM SURFACE-ZONE INCORPORATION OVER THAT FROM SUBSURFACE INCORPORATION					
	SUBSURFACE-ZONE INCORPORATION			FIXATION IN SUBSURFACE ZONE			SUBSURFACE-ZONE INCORPORATION			FIXATION FROM SURFACE-ZONE INCORPORATION OVER THAT FROM SUBSURFACE INCORPORATION		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
$\text{Ca}(\text{OH})_2$ . . . . .	320	3,250*	271	2,979	83.4*	260	3,310	1,148	2,162	60.5†	81.7	22.9‡
$\text{CaO}-\text{MgO}^{\ddagger}$ . . . . .	280	3,290*	231	3,059	85.7	300	3,270	1,215	2,055	57.6	1,004	28.1
$\text{CaO}-\text{MgO}^{\ddagger}$ . . . . .	280	3,290*	156	3,134	87.8	400	3,170	1,090	2,080	58.2	1,054	29.6
L.S. 10-20 . . . . .	3,340	230	78	152	4.3	1,720	1,850	506	1,344	37.6	-1,192	-33.3
L.S. 20-40 . . . . .	540	3,030	170	2,860	80.1	340	3,230	817	2,413	67.6	447	12.5
L.S. 40-80 . . . . .	80	3,490	211	3,279	91.8	0	3,570	1,098	2,472	69.2	807	22.6
L.S. 80-200 . . . . .	200	3,370	226	3,144	88.1	0	3,570	1,133	2,437	68.3	707	19.8
L.S. Comp. § . . . . .	1,300	2,270	51	2,219	62.1	680	2,890	900	1,990	55.7	229	6.4
Dol. 10-20 . . . . .	3,416	154	38	116	3.2	3,100	470	153	317	8.9	-201	-5.7
Dol. 20-40 . . . . .	1,740	1,830	105	1,725	48.3	1,240	2,330	486	1,844	51.6	-119	-3.3
Dol. 40-80 . . . . .	1,020	2,550	152	2,398	66.9	320	3,250	805	2,445	68.5	-47	-1.6
Dol. 80-200 . . . . .	300	3,270	207	3,063	85.8	220	3,350	1,013	2,337	65.5	726	20.3
Dol. Comp. § . . . . .	2,080	1,490	115	1,375	38.5	1,400	2,170	689	1,481	41.5	-106	-3.0

\* Assumption of theoretical carbonation.

† Calcined dolomite.

‡ Corresponding mixture of separately calcined CaO and MgO.

§ Equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

¶ Basis of  $\text{CaCO}_3$ -equivalence of addition.

chiefly as silicate complexes. The fixations, expressed as pounds and as per cent of the constant  $\text{CaCO}_3$ -equivalence of incorporations, are given in columns 4 and 5 for the surface zone and in columns 9 and 10 for the subsurface zone. The high-calcic and high-magnesian limes were considered as equivalent quantities of carbonates, as justified by previously reported results relative to the carbonation and fixation of  $\text{CaO}$  and  $\text{MgO}$  (5, 8, 12).

Carbonate  $\text{CO}_2$  results established the fact that neither zone enriched the carbonate content of the other; therefore no evidence was adduced to indicate movement of undisintegrated particles resulting from either frost action or gravity. Capillary lift of dissolved bases from the lower zone to the upper zone may be ignored; but it is certain that there was some passage of surface-zone incorporations of Ca-Mg and some enrichment of the alkali-earth content of the lower zone as a result of solution and leaching, though such specific fixations by the untreated lower zone are not shown. These could be established quantitatively only by ultimate analyses of both zones, and if the proportion between soil and treatment is considered, it is doubtful whether the time and labor incident to such analyses could be justified by the accuracy which might be anticipated for the results. Moreover, the fixed bases of both zones are within the feeding range of most plants whose roots do not penetrate below the surface soil. Studies are being made, however, of the increase in replaceable bases derived from each incorporation in the treated zone, as compared to that translocated to the adjacent untreated zone.

#### *Fixation from surface-zone incorporations*

*Burnt limes:* The results show that the average fixation from the three types of burnt lime was 85.6 per cent of the addition, when the incorporations were made in the surface zone. When this averaged fixation for the caustic group is supplemented by the small carbonate-residue average, we find that 93.8 per cent of the incorporation is still present after 4 years of fallow exposure. The fixation from  $\text{Ca}(\text{OH})_2$  and that as an average from the two  $\text{CaO}-\text{MgO}$  incorporations were, respectively, 11 times and 16 times as great as the corresponding enhancements in Ca-Mg outgo. Since the three carbonate residues were practically the same, it is evident that the greater retention shown for the two magnesian limes was due either to the stability of the absorbed magnesium in the upper zone, or to a greater removal of the magnesium salts from the leachings during their movement through the untreated lower zone.

*Limestone:* The meagre fixation of the 10-20-mesh limestone separate in the surface zone is in accord with, and less than, the small disintegration of this separate. With such slight disintegration and fixation, together with the previously reported (7) minimum influence exerted by this separate upon outgo of nitrates and sulfates for the 4-year period, it would seem that no commercial value should be claimed for limestone particles of this coarseness, when they are incorporated in the surface zone without subsequent mechanical transfer to lower depths. The 20-40-mesh product, however, gave a decidedly larger

fixation and also outgo, both values having approximated the corresponding ones from  $\text{Ca}(\text{OH})_2$  and the 40-80-mesh and the 80-200-mesh separates. The composite of limestone separates gave results less than, though most nearly approaching, those from the 20-40-mesh separate. The fixation from each surface-zone incorporation was very much greater than the corresponding enhancement in Ca-Mg outgo. Excluding the slightly disintegrated 10-20-mesh product, the limestone group gave an average fixation of 80.5 per cent of the incorporations, the balance being accounted for by a leaching average of 4.6 per cent and a carbonate-residue average of 14.8 per cent. The average

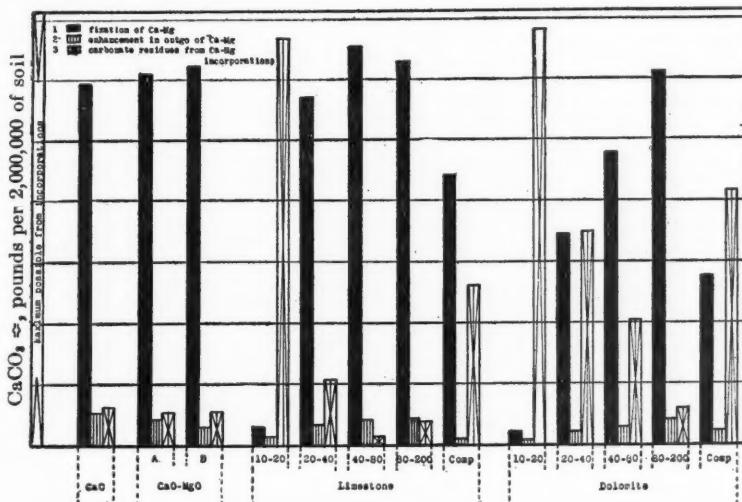


FIG. 1. THE FATE OF SURFACE-ZONE INCORPORATIONS OF  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , AND LIME-STONE AND DOLOMITE SEPARATES IN A LOAM SOIL, AT A CONSTANT  $\text{CaCO}_3$ -EQUIVALENCE OF 3570 POUNDS (2000 POUNDS  $\text{CaO}$ ) PER 2,000,000 POUNDS OF SOIL, AS ACCOUNTED FOR BY FIXATION, LEACHING AND CARBONATE RESIDUES AFTER 4 YEARS' EXPOSURE

Sum of 1, 2 and 3 equals the amount of Ca-Mg incorporated  
Burnt  $\text{CaO}-\text{MgO}$ : A—calcined dolomite; B—separately calcined  $\text{CaO}$  and  $\text{MgO}$

Ca-Mg fixation from the 20-40-, 40-80-, and 80-200-mesh limestone separates was 15.3 times as great as the corresponding average increase in Ca-Mg outgo through leaching. The average fixation of 86.7 per cent from those 3 separates was 3.3 per cent greater than that from the high-calcic lime.

*Dolomite:* The dolomite separates gave the same relative order in fixation as those of limestone, but the difference between the fixation from each separate and that from the one next in fineness was more marked than in the limestone series. The fixation from each dolomite separate was also less than that from its corresponding limestone separate, but the disparities between corresponding

limestone and dolomite fixations decreased with increase in fineness. This again emphasizes the fact that the more insoluble the limestone—and dolomites are generally less soluble—the finer should be the material applied. The fixation resulting from each surface-zone incorporation of dolomite was decidedly greater than the amount leached and, in the case of the 40-80-mesh and 80-200-mesh separates, the amount of dolomite fixed was materially greater than the undisintegrated residues. Disregarding the 10-20-mesh separate, which suffered very little disintegration, the dolomite group gave an average fixation 14.8 times as great as the enhancement in Ca-Mg outgo from the same group. The maximum fixation from the 80-200-mesh material was practically the same as that from the two magnesian limes.

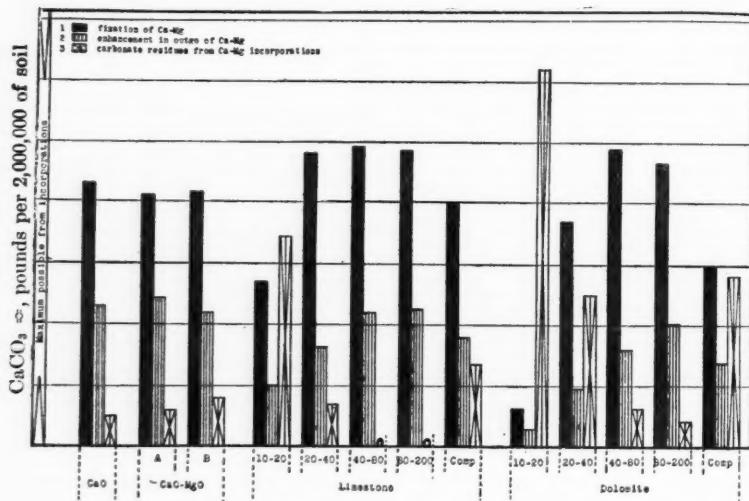


FIG. 2. THE FATE OF SUBSURFACE-ZONE INCORPORATIONS OF  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , AND LIMESTONE AND DOLOMITE SEPARATES IN A LOAM SOIL, AT A CONSTANT  $\text{CaCO}_3$ -EQUIVALENCE OF 3570 POUNDS (2000 POUNDS  $\text{CaO}$ ) PER 2,000,000 POUNDS OF SOIL AS ACCOUNTED FOR BY FIXATION, LEACHING, AND CARBONATE RESIDUES AFTER 4 YEARS' EXPOSURE

Sum of 1, 2 and 3 equals the amount of Ca-Mg incorporated  
Burnt  $\text{CaO}-\text{MgO}$ : A—calcined dolomite; B—separately calcined  $\text{CaO}$  and  $\text{MgO}$

#### *Fixation from subsurface-zone incorporations*

**Burnt limes:** In the subsurface group the fixations from the three limes were close to 60 per cent, or less than the average of 68.4 per cent from the three closely agreeing finer limestone separates, as accounted for by the larger average outgo and also larger carbonate residues from the three burnt limes. The carbonate residues from burnt limes are due to the unavoidable lumping during

incorporation with the soil and the resultant protective effect of  $\text{CaCO}_3$  coatings over the small lumps, which has been previously stressed (13, p. 414).

**Limestone:** The minimum fixation of limestone came from the 10-20-mesh separate. The disintegrative action of the subsurface zone was of such intensity as to bring the three limestone separates, 20-40, 40-80- and 80-200-mesh, to the same level by the end of the 4-year period. The fixation from the limestone composite again agrees most closely with that from the 20-40-mesh separate. Not including the 10-20-mesh separate, the average limestone fixation was 2.36 times as great as the corresponding enhancement in Ca-Mg content of leachings, whereas a corresponding proportion of 2.40 to 1 obtains from similar averages for the three finer limestone separates. Each of the three finer limestone separates gave a fixation in excess of that from the high-calcic lime.

**Dolomite:** The resistance of the dolomite to disintegration is again well demonstrated by the minimum fixation of the 10-20-mesh separate. The influence of fineness in overcoming this resistant property of dolomite is further demonstrated by the marked increase in fixation from the 20-40-mesh separate over that from the 10-20-mesh product before the end of the 4-year period and also in the final approximation of the 40-80-mesh and 80-200-mesh results to those from the corresponding limestone separates. Not including the 10-20-mesh separate, which underwent such slight disintegration, the dolomite group fixation was 2.71 times as great as the average increase in Ca-Mg leachings, whereas for the 20-40, 40-80, and 80-200-mesh average a corresponding value of 2.88 obtains. The fixation from each of the two finer dolomite separates was greater than that of each of the two high-magnesian limes in the deep incorporations.

#### *Surface-zone versus subsurface-zone incorporations*

The variations between the fixation resulting from surface-zone and subsurface-zone incorporations are given in columns 11 and 12 of table 1 and are shown graphically in figure 3. The fate of each incorporation, as accounted for by non-carbonate fixation, leaching, and carbonate residual, has been given in detail in figures 2 and 3 for upper- and lower-zone incorporations, respectively.

If the two zones were of equal effect in bringing about disintegration of carbonates, it would follow that the greater fixation and conservation of bases would be found where the incorporations were made in the surface zone, for the lower acid zone would retain some of the bases carried by the leachings from the enriched upper zone. The two zones, however, developed distinctively different capacities, or rather speeds, for carbonate disintegration. This greater decomposition in the lower zone may be accounted for by increase in mineral "acidoids" after placement, as influenced by greater bacterial activities in the usually more moist second 4 inches (1, 4) and by parallel enhancement in Ca-Mg outgo. Frear (2) was of the opinion that the surface zone is the most

acid stratum of the surface soil, but if true as a periodic variation in cultivated soils, such a variation did not prevail when the present soil was placed, because of thorough mixing. A 1:5 aqueous suspension of the air-dried loam, sampled when placed and then stored for 4 years, gave an electrometric pH value of 5.96, as against pH values of 6.08 and 6.18 for the upper and lower zones of the controls after 4 years of leaching. However, after an extraction immediately preceding the determination (14), these three samples—reserve and upper and lower zones which had been exposed for 4 years—gave corresponding pH values of 7.04, 6.95, and 6.63.

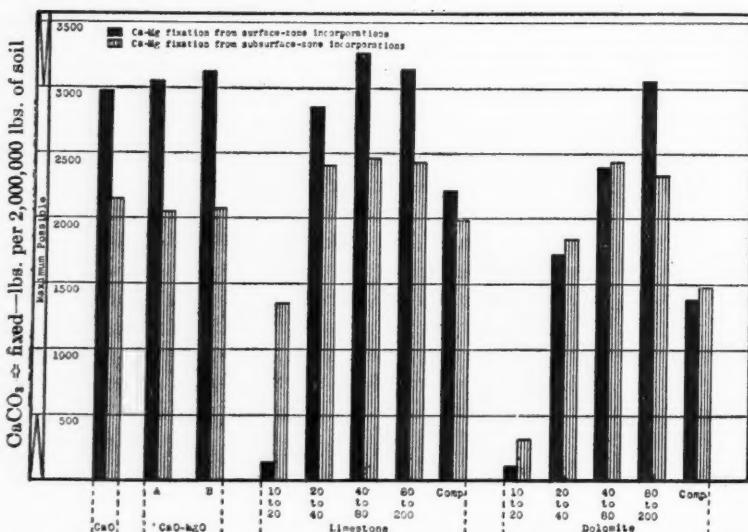


FIG. 3. INFLUENCE OF ZONE OF INCORPORATION UPON THE FIXATION OF CALCIUM-MAGNESIUM FROM  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , AND LIMESTONE AND DOLOMITE SEPARATES IN A LOAM SOIL, AT A CONSTANT  $\text{CaCO}_3$ -EQUIVALENCE OF 3570 POUNDS (2000 POUNDS  $\text{CaO}$ ) PER 2,000,000 POUNDS OF SOIL AFTER 4 YEARS' EXPOSURE—TERMS OF  $\text{CaCO}_3$  PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS

Burnt  $\text{CaO}-\text{MgO}$ : A—calcined dolomite; B—separately calcined  $\text{CaO}$  and  $\text{MgO}$

The 2-zone influence at work in the case of the upper-zone incorporations, as compared with the lower-zone influence alone in the case of the deep incorporations, is responsible for material differences in Ca-Mg outgo. The greatest leachings from the subsurface-zone incorporations of limestone came from the two finer separates which suffered complete disintegration, so that it would seem that the lower zone of greater moisture content and greater bacterial activities also gave greater hydrolysis of the absorption complexes.

*Burnt limes:* The average fixation from the three most soluble and active

burnt lime materials was 85.6 per cent of the addition, where both zones restrained outgo of bases, as against a non-carbonate retention of only 58.8 per cent for a similar average where the bases passed directly from the lower zone of incorporation and through the sand filters.

*Limestone:* When incorporated in the surface zone, each of the three finer separates and the composite of limestone showed fixation in excess of that from its analogue in the lower zone. The average of these four fixations in the surface zone was 2876 pounds, or 80.6 per cent of the addition, as against 2328 pounds and 65.5 per cent, respectively, for corresponding averages from incorporations made to the lower zone. In the case of the 10-20-mesh limestone separate, the carbonate disintegration in the lower zone was 8.04 times that in the upper zone and a minus value of 33.3 per cent is obtained in the last column. The carbonate disintegration in the surface zone was only 230 pounds, which was the maximum amount that the subsurface zone could stop for the 4-year period, whereas the disintegration in the subsurface zone was 1850 pounds—506 pounds of which passed into the leachings. But with the finer separates, where the extent of surface-zone disintegration equalled or approached that in the subsurface, the retentive property of the subsurface zone is definitely registered. This untreated surface soil stratum lying below the upper treated zone, thus shows the retardative tendency which was exerted more intensely by its subsoil (9) in stopping large amounts of Ca-Mg from heavy additions, the stoppage by the surface soil, however, being without basic interchange (10). The smallest excess of fixation exerted by both zones over that in the subsurface zone alone is shown for the composite, with the greater values for the separates of lesser size. The calculated average for the composite shows a value of 7.2 per cent, as against the determined value of 6.4 per cent.

*Dolomite:* The same observations made for the minus value for the 10-20-mesh limestone in columns 11 and 12 apply also to 4 of the 5 dolomite additions, which were more extensively disintegrated in, and more extensively leached from, the lower zone. But, the 80-200-mesh dolomite was of such fineness as to offset the less rapid disintegration in the surface zone, so that the carbonate residuals for the upper and lower zones were concordant and only 300 pounds and 220 pounds, respectively. In this case the disintegrated fraction, subject to leaching from the surface zone and through the subsurface zone, amounted to 3270 pounds, as against the 3350-pound disintegration which was subject to outgo from the subsurface zone alone. However, the outgo from the latter was 806 pounds in excess of that from the former. The 20.3 per cent 80-200-mesh dolomite fixation is, therefore, practically identical with that of 19.8 per cent for the corresponding limestone separate.

The Ca-Mg outgo from the surface-zone incorporations of 40-80-mesh dolomite was only 55 pounds less than that from the 80-200-mesh dolomite separate, as a result of the equalizing action of the untreated lower zone; but the carbonate disintegration from the 80-200-mesh separate was 720 pounds

in excess of that from the 40-80-mesh separate. A decided difference in the speed of disintegration of these two dolomite separates is thus manifested. However, this difference in speed of disintegration was greatly minimized in the more active lower zone where the differences between their respective carbonate disintegrations and leachings were only 100 pounds and 208 pounds, respectively. With this lack of disparity between outgo of Ca-Mg from lower-zone incorporations of these two dolomite separates, together with the corresponding small and concordant carbonate residuals, it is apparent that only small differences obtained at any time in the amounts of absorbed bases subject to hydrolysis and leaching from the lower zone. As a result of the difference in speed of disintegrations of the two finer separates in the surface zone and the near-equal disintegration in the lower zone, the upper-zone incorporation of the more rapidly disintegrated 80-200-mesh dolomite separate shows a fixation 20.3 per cent in excess of that found for the same separate in the lower zones, as against a minus value of 1.6 per cent in the case of the less rapidly absorbed 40-80-mesh separate.

The fixation from the surface-zone incorporation of  $\text{Ca}(\text{OH})_2$  was 22.9 per cent in excess of that from the lower-zone incorporation, on the basis of the 3570-pounds  $\text{CaCO}_3$ -equivalent addition. This difference is of practical and economic importance. The higher corresponding difference of 28.85 per cent as an average from the  $\text{CaO}-\text{MgO}$  additions, reflects the greater insolubility of the magnesium absorption-complexes. Greater fixations from surface-zone incorporations are also shown for the three finer limestone separates and composite; but the reverse is true of the 10-20-mesh separate. This is accounted for by the fact that the disintegration of the 10-20-mesh separate in the subsurface zone was 8.03 times that of the surface-zone incorporation, whereas their respective ratios between enhancement in outgo and fixation were 1.95:1 and 2.65:1. The curves obtained by plotting Ca-Mg outgo from limestone and dolomite against the fineness of their separates give a line gradually rising with fineness, for both materials and for both zones of incorporation. However, the curves for both materials are more nearly horizontal lines, in the case of the surface zone incorporations. This illustrates the equalizing effect exerted by the lower untreated zone, as offsetting variations in magnitude of fixations and in residual carbonates in the upper zone. In the lower zone there appears a good correlation between fixation and Ca-Mg outgo. This may be taken as indicating that the hydrolysis of absorbed materials served to furnish most of the leached Ca-Mg. Corresponding curves for carbonate residuals give V-shape conformations, with 10-20-mesh separates and composites as terminals. The sides of the curves are much more precipitous in the case of the upper-zone incorporations. The apex of the triangle was more widely spread for the limestone than for the dolomite in the upper-zone incorporations. In the comparison between zones there was a decidedly greater spread at the apex for limestone and for dolomite, in the lower-zone carbonate residues. Disregarding all variations in speed of disintegration and

extent of leachings, the 13 equivalent incorporations in the surface zone gave an average  $\text{CaCO}_3$ -equivalent fixation of 2269 pounds, as against 1952 pounds from subsurface-zone incorporations. If the two 10-20-mesh products be eliminated, the other 11 incorporations give corresponding fixations of 2658 pounds and 2156 pounds, or 74.5 per cent and 60.4 per cent of incorporations for the upper and lower zones of incorporation, respectively. The entire 13 incorporations gave an average fixation 14.6 times as great as the average enhancement in Ca-Mg outgo for the upper-zone incorporations. Excluding the two 10-20-mesh separates, the ratio of fixation to outgo is 15.4:1. Corresponding ratios of 2.29:1 and 2.28:1 are obtained for the 13 separates and 11 separates, respectively, in the case of subsurface-zone incorporations.

As a general conclusion it is established that the surface-zone incorporation of each treatment resulted in a greater Ca-Mg fixation, though less rapid carbonate disintegration, than that found for the corresponding incorporation in the subsurface zone. Conversely, ultimate disintegration and outgo of Ca-Mg from each incorporation were both greater when made in the lower zone. The full depth of soil was effective, however, in the case of the upper-zone incorporations, the upper zone serving as a disintegrating and retaining medium and the lower mainly, if not entirely, as an absorptive stratum for the outgo of Ca-Mg from the overlying treated zone. On the other hand, only the lower zone, or only one-half of the full depth, functioned to effect disintegration and to fix the disintegrated fractions of the incorporations.

#### SUMMARY

Data are given to show the fate of equivalent  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , and limestone- and dolomite-separate incorporations in the upper and lower zones of a loam soil after 4 years of outdoor exposure, as accounted for by the final amounts of non-carbonate fixations, leachings, and carbonate residues.

After extraction of soluble salts the lower zone of the untreated and initially uniform soil showed an acidity in excess of that of the upper zone as the result of 4 years of leaching without cultivation.

The Ca-Mg fixation, leachings, and carbonate residues from  $\text{Ca}(\text{OH})_2$ , and two  $\text{CaO}-\text{MgO}$  incorporations in the surface-zone are comparable, whereas their fixations and outgo do not differ greatly from the corresponding ones from the 20-40-, 40-80-, and 80-200-mesh limestone, and the 80-200-mesh dolomite separates. The average of fixations from those 7 incorporations is 3074 pounds, or 14.07 times the 210-pound corresponding loss by leaching. Both 10-20-mesh separates gave only slight fixation and acceleration in Ca-Mg outgo. Each composite of separates agreed most closely with its respective 20-40-mesh separate in extent of fixation. The average fixation of the 13 surface-zone incorporations was 2269 pounds, or 14.6 times a similar 155-pound average for outgo.

In the subsurface-zone series the fixation, leaching, and residual carbonate

results from  $\text{Ca}(\text{OH})_2$  and the  $\text{CaO}-\text{MgO}$  incorporations show concordance. The more slowly though completely disintegrated limestone separates of 40-80-mesh and 80-200-mesh fineness show greater fixation and approximately the same outgo as the caustic group. Though not completely disintegrated, the 20-40-mesh limestone and the 40-80- and the 80-200-mesh dolomite gave ultimate fixations in excess of—and leachings less than—the average from the caustic group.

In general the limestone separates were more extensively fixed than their corresponding dolomites, especially in the coarser materials. Except for the 10-20-mesh separates the disparities between limestone and dolomite fixations were not so great as those between leachings and carbonate residues.

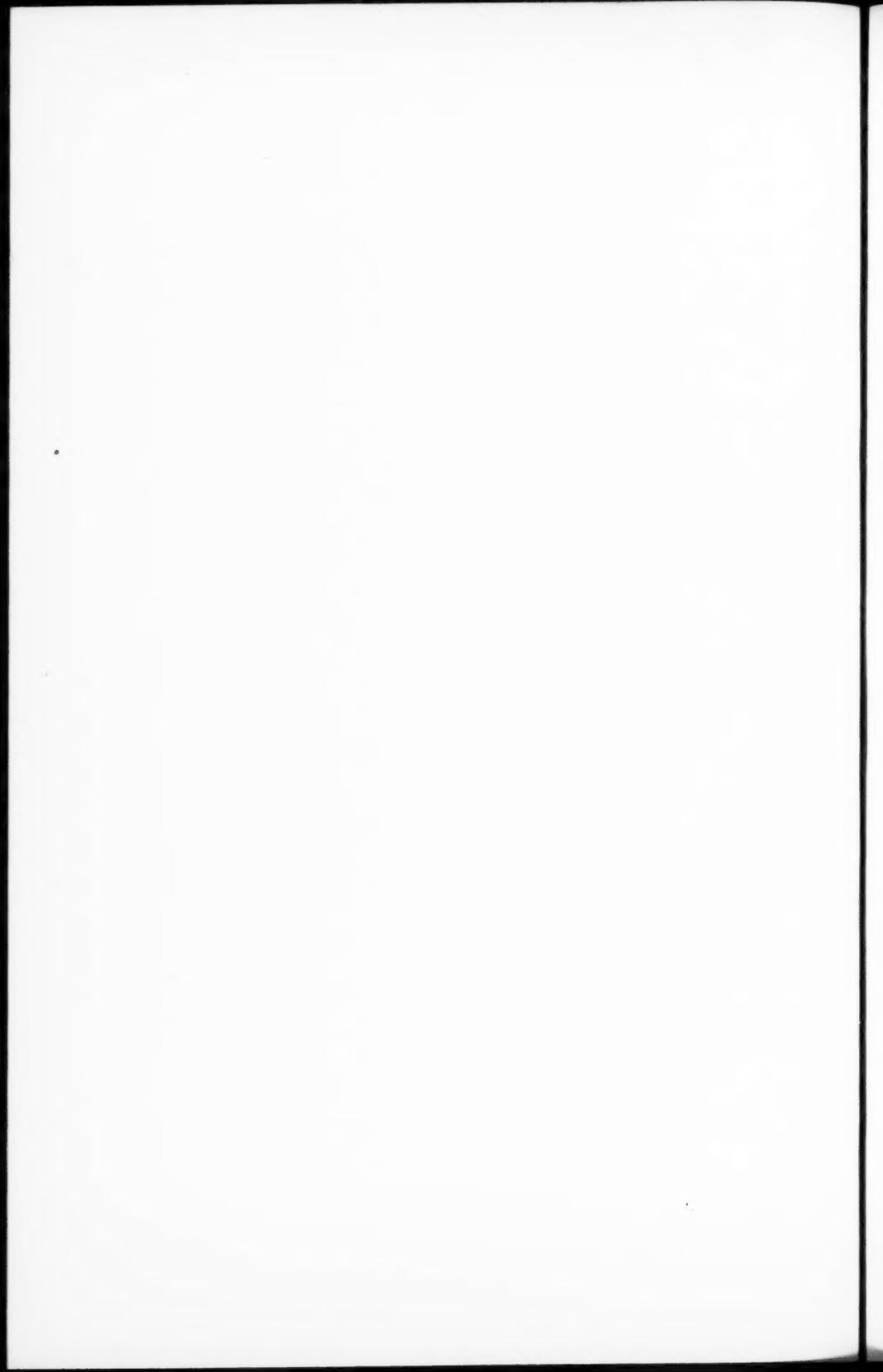
The ratios of fixation to outgo for the 13 incorporations were 14.6:1 for the upper zone and 2.29:1 for the lower zone. The supplementary effect of the untreated lower zone served to increase greatly the amount of Ca and Mg fixed from surface-zone incorporations of  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO}-\text{MgO}$ , and all of the limestone incorporations, with the exception of the 10-20-mesh separate. The lesser fixation of Ca-Mg in the lower zone is reflected in a correlative increase in outgo. The limestone separates were more extensively disintegrated in the lower zone, but with the exception of the 10-20-mesh separate, the fixation from subsurface-zone incorporations was less than that from incorporations in the upper zone. In the dolomite group the combination of greater disintegration and enhanced outgo gave greater fixation from subsurface-zone incorporations for the composite and all separates except the 80-200-mesh, the exception being attributable to the more rapid disintegration of this separate, as indicated by comparisons of its carbonate-residues and leachings with those of its surface-zone analogue and those of the 40-80-mesh separate from the two zones.

Practical adaptations of the findings are (a) The coarser the separate the deeper should be the incorporation to insure disintegration; (b) Dolomite should be ground finer than limestone; (c) a 20-40-mesh product may be considered as about equivalent to an average 10-mesh product; (d) surface-zone incorporations will give the greatest conservation of alkali-earths.

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# THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": I. INTRODUCTORY AND HISTORICAL<sup>1</sup>

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## INTRODUCTION

The organic matter commonly found in the soil is distinctly different in composition from the tissues of plants and animals which are added to the soil, either in the form of stable or green manures or as various plant and animal residues. The soil organic matter is largely secondary in nature: it consists, on the one hand (*a*) of the constituents of plants and animals which have been introduced into the soil and which are undergoing decomposition, (*b*) of various intermediary products which have been formed under certain environmental conditions and which may be decomposed further, (*c*) of substances which resist decomposition and which may persist in the soil for a considerable time; and on the other hand, of a number of substances that have been synthesized by the numerous groups of microorganisms which, as living and dead cells and as cell derivatives, form the soil population. The soil organic matter is thus found to be genetically a heterogeneous mass of substances, undergoing constant change in composition. It approaches a condition of homogeneity when its composition reaches a certain stage of equilibrium and when it becomes more or less incorporated in the soil. This homogeneous mass is usually spoken of as "humus."

Just what this "humus" is and how it originates in the soil is still largely a matter of conjecture, although various theories—some based on experimental facts—have recently been submitted. These tend to explain the origin if not the nature of this "humus." There is a complete lack of uniformity concerning the very usage of the term "humus." Some investigators (largely European) use the term "humus" to designate the total organic matter in the soil. Frequently no attempt is made to differentiate between the organic matter added to the soil in the form of various organic residues, and the soil organic matter itself, which is distinctly different in composition and decomposes only with great difficulty. The terms "humus" and "humic substances" are often used to designate the dark colored materials formed from plant and animal tissues, after these have become decomposed (the exact nature of the process of decomposition remaining practically unknown) and incorporated into the soil.

<sup>1</sup> Paper No. 276 of the Journal Series, New Jersey Agricultural Experiment Station Department of Soil Chemistry and Bacteriology. This is the first of a series of five papers dealing with soil "humus."

This process is usually termed "humification" and the organic matter thus formed and having become a part of the soil is spoken of as "humified," in contradistinction to the "unhumified" portion of the original organic matter added to the soil. The soil "humus" is sometimes [König et al. (121), Gehring (81)] differentiated as total "humus," obtained by multiplying the carbon content of the soil by an arbitrary figure (1.75), and available "humus," obtained by the action of some reagent, such as potassium permanganate or concentrated hydrogen peroxide, upon the soil organic matter.

Some investigators (largely American workers) apply the term "humus" to that part of the soil organic matter which is soluble in alkalies. Still others limit the use of this term to that part of the alkaline extract which is precipitated by acids [Page (168), Beckley (22)].

A similar variety of usages applies to the term "humic acid." Some [Loughridge (136)] consider "humus" to be present in the soil as a compound of "humic acid" and lime. When the latter is adsorbed by lime-loving plants, such as legumes, the "humus" is left in the soil as "humic acid." "Humic acid" is often considered as that part of "humus" which is extracted by alkalies. In some instances the term is limited to that part of the alkaline solution, which is precipitated by acids. S. Oden (162) designated by the term "humic acid" that part of the acid precipitate which is insoluble in alcohol, the soluble portion being the "hymetomelanic acid" of Hoppe-Seyler (106). A number of "humic acids" have thus been separated, largely according to their solubility in different reagents, and a number of formulae have been suggested for various "humus" and "humic acid" preparations (154, 98). Few of these are based on facts obtained by experiments and in the very few instances where claim could be laid to the existence of more or less definite chemical compounds in the soil (162), no attempt was made to learn just how these "humic acids" have originated in the soil and how they can be further decomposed. No attempt has ever been made to learn whether the "humus" of peat soils is the same as that of normal cultivated soils, although this has tacitly been assumed.

When fresh organic matter, in the form of plant or animal materials, is added to normal soil, some of the constituents of this organic matter begin to undergo rapid decomposition, in which a large number of microorganisms constantly present in the soil participate. The nature of these organisms depends on the nature of the soil (mechanical and chemical composition, physical condition) and on environmental conditions (moisture content, reaction and aeration, and presence of available minerals). The decomposition processes can best be followed by measuring one of the final products of the reaction, such as the evolution of carbon dioxide. The rapidity of decomposition depends on the nature of the organic matter, on the organisms active in the process, and on soil environmental conditions. After the rate of decomposition has reached a maximum, it begins to diminish until it reaches a certain condition of "equilibrium," if the soil is kept under the same experimental conditions. The carbon dioxide liberated accounts for only a part of the carbon of the

organic matter added to the soil. Another part will persist in the soil and will tend to become an integral constituent of the soil organic matter. The condition of "equilibrium" becomes established only when the readily decomposable constituents of the natural organic matter (sugars, starches, pectins, celluloses, proteins, amino acids) added to the soil have disappeared and only those constituents which are less readily acted upon are left. These and certain synthesized substances, both of which decompose only slowly, contribute to the soil organic matter. Once the early phases of decomposition, equivalent to the so-called processes of "decay" and "fermentation" have passed, the residual and synthesized organic matter and the organic matter of the soil itself (the soil "humus") undergo only a slow transformation, with the result that a moderate but constant stream of  $\text{CO}_2$  is being liberated and probably also, in a parallel manner, a constant stream of ammonia, which is changed, under favorable conditions, to nitrate (239).

Schreiner and Shorey (204) calculated that the average content of organic matter in the soils of the United States is 2.06 per cent and of the subsoils 0.83 per cent. This organic matter contains the essential element nitrogen and some of the minerals (phosphorus and sometimes potassium), which must be liberated in an available form, before they can be utilized again by cultivated plants. This can take place only when the soil organic matter is decomposed by the soil microorganisms. The decomposition is very slow, as can be readily demonstrated by placing a quantity of soil under favorable environmental conditions and measuring the rate of decomposition either by the evolution of carbon dioxide or by the accumulation of ammonia and nitrate nitrogen. Since the ratio between the carbon and nitrogen content of the organic matter in normal cultivated soils is more or less constant, approaching 10 to 1 (77, 211, 38, 236), the evolution of  $\text{CO}_2$  will be accompanied by a liberation of available nitrogen. The decomposition of the organic matter that has become a part of the soil and that will be referred to as "soil organic matter" is comparatively very slow because only limited and slow growing groups of microorganisms are capable of attacking it, as will be shown later. The decomposition processes can be greatly hastened in nonacid peat soils by draining, in acid peat soils by draining and liming, and in acid soils by liming. These treatments favor the development of the particular organisms which are capable of decomposing the "soil organic matter" and liberate the elements necessary for the nutrition of higher plants.

A knowledge of the formation, nature, and decomposition of the soil organic matter is the most important and most outstanding need of soil science today.

The earlier investigators assumed that soil organic matter is formed from plant and animal substances by chemical agencies, especially by the action of atmospheric oxygen and of water. Natural organic substances added to the soil were known to contain considerable quantities of various sugars and higher carbohydrates, proteins, and other nitrogen compounds, which are readily acted upon by microorganisms in the presence of sufficient moisture and at a

favorable temperature; it was also known that the soil harbors numerous microorganisms capable of bringing about the decomposition of those substances. Upon this knowledge was based the assumption that the soil microorganisms take an active part in the transformation or "humification" of the organic matter added to the soil (103, 102), but no experimental evidence was offered to throw light upon the various processes involved. The accumulation and the persistence of considerable quantities of organic matter in the soil without further rapid decomposition indicate that, although the natural organic matter contains substances readily acted upon by microorganisms, the organic matter that is formed in the soil is not very readily acted upon. This can be due to one or more of the following phenomena:

- (a) the natural organic matter contains some substances which are readily decomposed and some which resist decomposition;
- (b) substances are formed from certain ingredients of the organic matter added to the soil which resist further decomposition;
- (c) the activities of microorganisms bring about a synthesis of substances which persist in the soil.

The chemist treated the soil with various reagents, usually acids and alkalies, alcohols, ether, pyridine, and other solvents and succeeded in isolating various complex bodies from the soil organic matter, but he gained only a limited amount of information concerning the origin of these bodies in the soil and their relation to the other organic and inorganic compounds. He has often compared the soil organic matter, or the so-called "natural humus" obtained by treating the soil with alkalies, with the dark colored substances produced artificially in the laboratory by the action of mineral acids upon carbohydrates ("artificial humus") and found in most instances no seeming differences between the two, forgetting entirely the fact that the soil harbors millions of organisms which are capable of breaking down sugar completely within a very short time and that mineral acids as such are absent in normal soils. The chemist was often led to conclude that the substances found in the soil are largely a result of the method of their extraction and in some instances no difference was even found between the "humus" of the soil and that of the natural organic materials added (86). The physicist and the physical chemist were interested in the soil organic matter largely as a physico-chemical colloidal complex; considerable information was thus contributed to the physical properties of this complex, especially in regard to the processes of combination of this complex with various bases ("adsorption" phenomena) but very little was contributed to our knowledge of the origin and nature of the complex (167). The microbiologist recognized early the numerous processes involved in the transformation of organic matter in the soil, the numerous organisms taking part, and the various complex reactions brought about, but he limited himself to gaining first information on the transformation of the simple ingredients of the natural organic matter, under controlled conditions, by pure or mixed cultures of microorganisms, without attempting to solve the more complex problems. The agronomist recognized the important rôle that soil organic

matter plays in soil fertility processes, but without knowing its nature and the conditions of its formation and accumulation he had no means of controlling its quantity and quality. That there is a difference in the quality of the soil organic matter and in the manner in which it decomposes is readily recognized when two different soils containing the same amounts of organic matter are compared; when the evolution of carbon dioxide or the accumulation of ammonia and nitrate nitrogen is used as an index, distinctly different results between the two soils may be obtained. Falck (66) clearly demonstrated that the nature of transformation of organic matter in forest soils and the type of soil resulting depend entirely on the organisms taking part in the decomposition and upon the environmental conditions influencing these processes.

The lack of sufficient knowledge concerning this problem which has attracted the attention of numerous investigators, including some of the most brilliant chemists, is due to its great complexity and to the faulty methods of investigations. The various constituents of the plant and animal tissues introduced into the soil, including fats and waxes, simple and complex carbohydrates, lignins, glucosides, proteins and their derivatives, alkaloids, pigments, tannins, phenol derivatives, and resins, are decomposed partly or completely, yielding a complex mass of dark colored substances, in which the identity of most original materials is lost. Instead of studying the genesis of "humus," attempts were made to determine its chemical nature. Various compounds have been isolated (204) from this complex mass, by different chemical manipulations; but the possibility has not been excluded that some of these at least were not present in the same form in the soil organic matter itself, but have been split off in the process of preparation. Nearly all the investigations dealing with the nature of the soil organic matter were limited to the soil itself, already formed, and few attempts have been made to study the course of its formation. As a recent investigator (231) expressed it, the need has been felt in

1. studying conditions under which the process of "humus" formation proceeds in one direction or another;
2. establishing what organic constituents of the plant vegetation are the sources of soil "humus";
3. determining the agents of "humus" formation;
4. studying the chemical processes whereby the natural organic materials change into "humus";
5. determining the chemical nature of the "humus" itself.

A complete review of the earlier literature on the soil organic matter, especially in reference to the "humic acids" is given by Wollny (250), Baumann (18), Grafe (89), Löhnis (134), Trussov (231), Czapek (45), Ehrenberg (59) and Oden (162). Attention is directed here only to those contributions which materially advanced the understanding of the nature of the soil organic matter and of processes leading to its formation.

## HISTORICAL

*The nature of soil organic matter*

Achard (2) in 1786 was the first to use alkalies as solvents for the extraction of a brown substance from the soil. A similar substance was extracted by Vauquelin (234) from an elm tree (*Ulmus*) infected with fungi. This substance was found to precipitate when the alkaline solution was acidified; it was partly soluble in alkalies and could form compounds with bases. The term "ulmin" was applied to this substance by Klapproth (174). De Saussure (194) was the first to express the idea that soil "humus" originates from vegetable matter by "the combined action of air and water." A number of contributions on the subject of soil organic matter soon followed, including those of Einhof (63), Braconnot (35), Proust (174), Berzelius (31), in which these substances were referred to under the terms "ulmin" and "humin." Braconnot (37) extracted from the rotted material collected at the root cavities of an old tree a substance soluble in alkalies and precipitated by acids, in the form of brown-black flakes, which he believed to be identical with "ulmin." He found "ulmin" not only in the rotted organic matter but also in great abundance in peat and even in lignite. He stated that ulmin must doubtless form an important constituent of "terre d'ombre," but it could not be obtained from coal. Braconnot was the first to obtain artificial ulmin by treating various organic substances with mineral acids, and lignous material with potassium hydroxide. Boullay (34) suggested that many vegetable materials are changed into ulmin under various influences; he found it in soil rich in vegetable matter, in manure, and in great abundance in peat soils. Ulmin, because of its ready combination with alkalies and its precipitation by acids, was looked upon as the best fertilizer.

The term "humic acid" was first suggested by Dobereiner (51) and then used by Sprengel (217), for the organic substances extracted by alkalies from soils. Sprengel, in 1826, was the first to describe the preparation, nature, and properties of "humic acid" and of its salts. He distinguished between "mild humus" and "acid humus" or peat, the latter originating in places where bases are lacking. It is interesting to note that this work carried out just a century ago exceeds in the understanding of the subject, in the accuracy of manipulations, and in the differentiation of the soil organic constituents, many of the contributions to the same subject published within the last few years and the treatment accorded the subject in recently published texts. According to Sprengel (217), "humic acid" is formed in the "fermentation" and "decay" of plants, whereby the larger part of its carbon combines with atmospheric oxygen and water. Sprengel, and Braconnot (36) previously, obtained artificial "humic acid" by treating woody tissues and plant residues with potassium hydroxide solution, the oxidation of the product by atmospheric oxygen being considered an essential part of the process. Sprengel stated that in a soil poor in bases, free "humic acid" remains without decomposing further, giving to the soil its acid reaction, as in the case of peat soils; when the soil is rich in bases, humic acid is further decomposed to carbon dioxide and water. Sprengel prepared humic acid by extracting pulverized air-dry peat with dilute hydrochloric acid for 2 hours, in order to separate the bases from the humic acid, then washing the residue upon a filter with water; the peat was then extracted with ammonia water in a closed vessel and the dark brown solution was neutralized with hydrochloric acid, which resulted in a precipitate. This precipitate was found to contain ferric hydroxide and clay. It was redissolved in sodium carbonate and reprecipitated in the cold with hydrochloric acid. "Humic acid" thus prepared was found to contain only traces of ash; it was shining black, easily pulverized, and possessed a great absorptive power for water. When freed from hydrochloric acid, the moist humic acid dissolved to a limited extent in water, but when dried, it became insoluble in water. On electrolysis, it went to the positive pole, behaving like an acid. It formed insoluble compounds (humates) with salts of alkali earths and with heavy metals. It combined with bases liberating  $\text{CO}_2$  from carbonates. Calcium and magnesium

humates were readily decomposed. The "humic acid" formed compounds with clay and with iron; it also liberated free silicic acid from soluble silicates.

The study of the subject so well begun was not advanced further during the following decades; as a matter of fact the introduction of new names and various formulae only tended to confuse it.

Berzelius (31) distinguished the alkaline-soluble fraction of the soil organic matter or "humic acid" from the alkaline-insoluble portion or "humin." Malaguti (141) demonstrated that, as a result of the action of hydrochloric and sulfuric acids upon sugars, "humic acid" and "humin" are formed. The product contained 57.5 per cent carbon and was considered to be very similar to the natural "humic acid" studied by Sprengel. All acids, both organic and inorganic, even in very dilute solutions, were found to give, on warming with sugar, "humic acid." The subject was further extended by Berzelius' pupil Mulder (152, 153, 154) who added new names and attempted to suggest chemical formulae for brown and black organic substances, found in decomposing organic matter and in artificial preparations. Mulder's name is usually quoted more often in connection with these organic soil compounds than that of any other investigator, largely because, as pointed out by Baumann (18), he has not given credit to Sprengel in his well known text, where he has placed in the background Sprengel's work, in comparing it with his own. The various terms and designations proposed by Berzelius and Mulder for substances, whose very existence is doubtful—crenic acid, apocrenic acid, geic acid—have appeared since in the literature and have persisted until the present time. Mulder dried his preparations at 140°–195°C., where he no doubt obtained considerable decomposition. He suggested at first that the nitrogen is present in the "humic acids" as ammonia, but later he declared that it must be present there as a sort of protein. Both Berzelius and Mulder considered "humic acid" to be present in the soil not as a free acid, while "humus" substances in general were not considered as acids, but were believed to become acids as a result of the alkali treatment.

Hermann (98) separated the various "humus" compounds into those that are: 1. soluble in alkalies and precipitated by mineral acids; 2. soluble in alkalies and not precipitated by mineral acids; 3. insoluble compounds. Hermann, like Mulder, believed that humus compounds can absorb nitrogen from the atmosphere. The nitrogen content of the "humic" bodies varied according to the formulae proposed, as  $C_{32}H_{32}O_4N_2$  (4.5 per cent nitrogen) for "indifferent humus bodies," and  $C_{12}H_6O_4N_2$  (11.6 per cent nitrogen) for "peat iso-crenic acid" or acid precipitated not by mineral acids but by metallic salts in solution acidified with acetic acid. The names and formulae suggested by Berzelius, Mulder, and Hermann can hardly stand criticism, but these authors have definitely pointed out that the soil organic matter consists of a number of substances, some of which contain nitrogen and some of which are free from it. The system of separating these substances, based on the solution by alkalies and precipitation by acids, proposed by these investigators is still the best that is available at the present time.

Detmer (50) came to just the opposite conclusion, namely, that the substances, which are dissolved by alkalies from peat, from soil, or from "humus" prepared artificially from sugar, and which are precipitated by an acid from the alkaline solution are all made up of a single compound—"humic acid" of the formulae  $C_{68}H_{54}O_{27}$ . The nitrogen was believed to be present in this body in organic combination and was considered as an impurity. The only substance different from "humic acid" was "humin acid," which was soluble in water. By repeated solution in potassium carbonate and finally in water and by reprecipitation with boiling hydrochloric acid, the nitrogen content was reduced to a minimum (from 1 or 2 per cent to 0.179 per cent). The "humic acid" thus obtained was similar in its properties to the natural product, although less soluble. Detmer's results seemed to point to the fact that the "humic acids" prepared from peat soils, by extraction with alkalies and precipitation with acids, consist of a nitrogen-poor or nitrogen-free compound and a substance rich in nitrogen. The nitrogen content of the "ulmin" from brown peat was found to be 0.64 per cent, of

"humin" from black peat 1.01 per cent, of "humic acids" from garden or field soils 3.3 to 3.6 per cent.

Eggertz (57), however, considered nitrogen to be a constituent of the "humic acid" molecule; he believed that iron and sulfur (0.5 to 2 per cent) belong also to the molecular complex. When "humic acid" was determined by Grandea's (90, 91) method; namely, by washing soil with hydrochloric acid, treating with ammonia, and precipitating the ammoniacal extract with a mineral acid, phosphoric acid was always found in the extract. Eggertz, therefore, concluded that all constituents of the living cells form a part of the "humic acid" molecule. The natural "humic acids" could thus not be considered as definite chemical compounds of carbon, containing hydrogen and oxygen in the ratio of water; they could not be compared with the artificial substances prepared by boiling carbohydrates with acids and alkalies, for the latter lack both the nitrogen and the ash. The difference between the natural and artificial products has also been established by other investigators. Miklauz (147) found that natural "humic acids" contain less carbon than the artificial forms. According to Robertson, Irvine, and Dobson (184), the natural compound contains 1.71 to 2.47 per cent of methoxyl groups, whereas the artificial forms were found to contain 6.47 per cent methoxyl. According to Sestini (206), the natural "humic acids" also contain considerably more furfural than the artificial forms.

The nature of the artificial "humic acids" prepared from carbohydrates has attracted the attention of a number of investigators; it is sufficient to say, however, that these preparations were found to vary greatly in composition depending on whether they were prepared by the action of acids or alkalies, on the nature and concentration of the acid (dilute acids giving alkali-soluble "humic acids" and concentrated acids giving alkali-insoluble "humins") or alkali, and on the nature and concentration of the alkali used for extraction of the preparation. As these involve complex chemical reactions, which depend upon a number of factors, Baumann (18, 19) came to the conclusion that the chemical formulae suggested for artificial humic acids deserve as little consideration as the formulae suggested for the natural humic acids. One can understand, therefore, how little basis there is for comparison between the natural and artificial forms of "humus." The work of Sestini (206) and Fröh (80) on the morphology of the "humus" compounds tended to confirm the observation of Mulder that these substances change to "humic acids" only on contact with alkalies.

Notwithstanding the various suggestions to the effect that "humic acids" are complex bodies, Berthelot and André (30) made an attempt to characterize both artificial and natural "humic acids" as definite organic acids. The brown, almost insoluble precipitate obtained by boiling sugar with hydrochloric acid was considered not as a mixture of different substances, but as an anhydride of "humic acid," which gradually changes, under the influence of water, into the hydrate. The "humic acids" were found to form, with sodium and potassium, monobasic salts soluble in water and tribasic salts insoluble but later decomposed in cold water. These investigators definitely noted that "humic acids" do not absorb nitrogen from the atmosphere.

The attention of the investigators was thus centered not on the soil organic matter as a whole but only on one certain definite part which was differentiated from the rest by its solubility in alkalies. Some, like Sprengel, Boullay, Malaguti, and Mulder, considered this "humic acid" to be a definite chemical substance. Sostegni (216) extracted soil with boiling sodium hydroxide, then precipitated the "humic acid" from the alkaline solution with hydrochloric acid; the precipitate was redissolved in alkali, reprecipitated with acid, and washed with water. The purified substance when treated with 85 per cent alcohol was separated into two fractions, one soluble in alcohol and the other insoluble. The soluble part contained 62.2 to 63.7 per cent carbon and the insoluble part contained 57.5 to 57.8 per cent carbon. Similar ideas were expressed by Hoppe-Seyler (106), who designated the alcohol-soluble portion as "hymetomelanic acid." Oden (162) separated the soil organic matter into 4 complexes, on the basis of solubility in alkalies, in water, and in alcohol:

1. The substances which are insoluble in dilute alkalies, but which are gradually dissolved by fusing with strong alkalies; namely, "humus coal," "ulmin" or "humin."
2. Substances soluble in alkalies, precipitated by acids, and insoluble in alcohol; namely, "humic acid."
3. Substances soluble in alkalies, precipitated by acids, and soluble in alcohol; namely, "hymetomelanic acid" and "ulmic acid."
4. Substances soluble in alkalies and in acids; namely "fulvic acid." Hoppe-Seyler and Oden are the outstanding later workers who claim a definite chemical composition for the various organic complexes in the soil.

Strache and Lant (219a) suggested that the different humic acids may consist of the same chemical compound, accompanied by various impurities; and that for the present it may be advisable not to attach any special names to the compounds obtained by different methods of procedure. At the same time they suggest separating the natural compounds, produced from the decomposition of organic materials ("natural humic acid" soluble in alkali carbonate and precipitated in acids, "humin" insoluble in alkali carbonate but soluble on continued boiling with hot alkalies, "humus coal" insoluble even on continued boiling with strong alkalies) from the artificial compounds obtained by treatment with chemical reagents ("artificial humic acid" or "huminoid acid" soluble in alkali carbonate and precipitated by mineral acids, and "artificial humin" or "huminoid substances" similar to "natural humins").

Sestini (206) demonstrated the presence in "humic acid" of anhydrides and ethereal substances, and of hydroxyl and alkyl groups, in the form of furan and benzol derivatives. Miklauz (147) found that when first the waxes are removed from peat by ether and alcohol extraction, then the bases by hydrochloric acid extraction, the treatment of the soil with alkali gives "humic acids" of greater solubility in alcohol than otherwise; the alcohol-insoluble part gives also a greater pyridine-soluble fraction than otherwise. Preliminary treatment of the soil with hydrochloric acid was found to result in an increase in the carbon content of "humic acids." The preliminary treatment of the soil with alcohol and acid resulted in a loss of 33 per cent of the organic matter. All alcohol-soluble substances were also found to be soluble in pyridine. Schreiner and Shorey (203) finally succeeded in separating from the "humic acid" a series of organic compounds some of which are colorless and some colored. Some of these are probably obtained as a result of the action of the reagents employed on the complex soil organic matter. The idea of the complexity of the soil organic matter including the "humic acids" is well expressed in the summary of the complex by Shmook (208): (a) Humic acid is a nitrogenous body of an acid nature, the acidity being due both to its power of adsorption, as a result of the colloidal condition of the humic acid, and to the presence of COOH groups. (b) Humic acid contains a tri-valent benzol ring. (c) A large part of the nitrogen in the "humic acid" is in the form of protein combined in a physico-chemical manner with the other part of the substance. A small part of this protein is less firmly combined and can be extracted by neutral salts; the protein of the humic acid is characterized by a high content of amide nitrogen. (d) The elementary composition of the humic acid is C—61.84 per cent, H—4.21 per cent, N—3.28 per cent, O—30.67 per cent.

The chemical composition of the so-called humic acids was thus found to depend largely upon the laboratory method of preparation; namely, the nature and concentration of alkali and acid used, and the temperature and length of action of the reagents. There is no wonder, therefore, that no comparison could be made between the results obtained by different investigators. The chemical formulae suggested as a result of data obtained by elementary analysis could not lay claim to accuracy, since no chemically pure substance could be obtained by the use of alkalies as solvents. In summarizing the previous investigations on the "humus" bodies of the soil, Schreiner and Shorey (203) noted that "the most conspicuous feature of this work is the discordant results obtained for bodies bearing the same name and often obtained from the same source."

But while some investigators were attempting to learn the nature of the soil organic matter or of certain of its constituents, others were attempting to compare the "humus" content of the soil with its fertility. The statement made by Hilgard (101, 102) that "unhumified" organic matter does not nitrify and that, therefore, it is unavailable for plant growth, led to various determinations of the "soil humus," "humus ash," and "humus nitrogen," without even any attempt to learn what these substances are and how they originated. In most instances, the original method of Grandjean (90) or one of its numerous modifications was used. It is sufficient to cite the work of Loughridge (137), Alway (7, 9), Kelley and McGeorge (116), and C. B. Lipman (131). These studies were not very fruitful in consequences, and the method of making "humus" determinations is all but abandoned, at present. It is of interest to note here that Hilgard found that the narrower the carbon-nitrogen ratio of soil "humus", the less is the nitrogen need for the growth of higher plants.

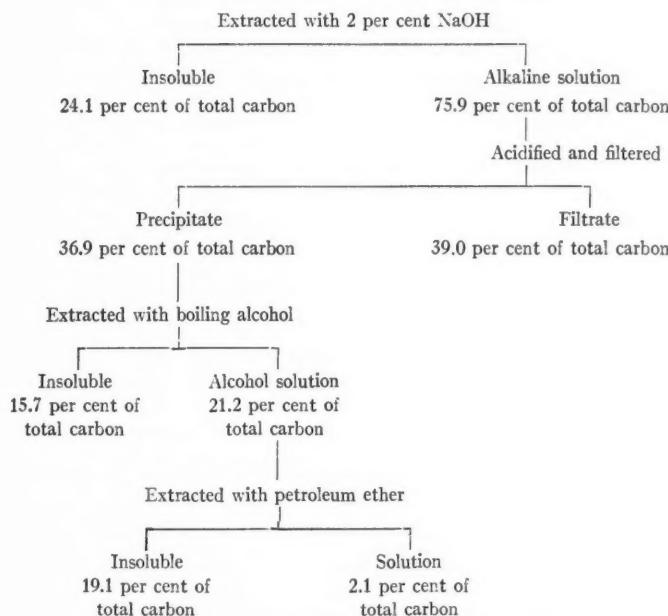
Several contributions to the subject of composition of the soil organic matter, made within recent years, however, have helped to obtain a better insight into the nature and origin of soil "humus." The work of Schreiner and Shorey (203, 204) and their collaborators of the Bureau of Soils is of especial importance in this connection. These investigators were the first to demonstrate definitely that the soil organic matter is not a single compound or a group of chemical compounds, but is made up of a large number of compounds. That the numerous plant constituents are in some mysterious way conglomerated into a single group of closely related bodies, the "humic acids," as assumed by a number of chemists and agronomists, is entirely disproved. Not only is the soil organic matter made up of a large number of compounds, but these are not closely related and belong to different classes, the types of compounds of soil organic matter being quite as varied as in plants or animals. Schreiner and Shorey have isolated from the acid filtrate, after the precipitate formed by the acidifying of the alkaline extract of soil has been removed, the following compounds: dihydroxystearic acid, picoline carboxylic acid, xanthin, hypoxanthin, cytosin, histidin, arginin, and pentosan; this mixture was usually classified as "crenic" and "apocrenic acid" and recently renamed by Oden as "fulvic acid." From the precipitate, usually referred to as "humic" or "ulmic" acid, they have isolated resin acids, resin esters, glycerides, paraffinic acid, lignoceric acid, agroceric acid, agrosterol, and phytosterol.

Schreiner and Shorey (204) suggested the method shown on page 133 for the separation of the different soil fractions.

Their statement that "the material known as soil organic matter is in the transition stage from the complex compounds of living organisms to the simple ultimate products" can be partly questioned, but they rightfully suggested, in the case of a number of compounds, that the synthesizing activities of the soil microorganisms probably have to do with their presence in the soil.

Notwithstanding this work, Oden (162) still adheres to the ideas of the earlier chemists that the soil organic matter consists of a few definite chemical compounds, classified into 4 groups, as shown above. In this respect, no advance has been made over the work of Berthelot and André (30) who divided the soil organic matter on a similar basis, but who found nevertheless that the different fractions vary in the relative amount of carbon to nitrogen, as shown in the following summary:

ORGANIC MATTER FRACTION	FRACTION CONTAINS PER CENT OF TOTAL CARBON	DISTRIBUTION OF THE TOTAL NITROGEN IN FRACTIONS <i>per cent</i>	CARBON NITROGEN RATIO
Part insoluble in cold alkalies.....	31.7	1.25	25
Part soluble in alkalies and precipitated by acid.....	27.6	1.52	18
Part soluble in alkalies, but not pre- cipitated by acids.....	40.7	3.9	10.3

*Soil containing 0.955 per cent organic carbon*

The fraction resisting the action of alkalies thus contains the least nitrogen, whereas the fraction that was made soluble by the alkali treatment and that cannot be precipitated by acids, contains most of the nitrogen. The composition of the "humic acid" or that part of the "humus" which is soluble in alkalies, but insoluble in acids, was as follows: C—55.2, H—6.8, N—3.9, O—34.1.

"Humic acid" was considered by Oden (161, 162) as a definite chemical complex. In differentiating between "humic compounds" and "humic acids," Oden defined the first as "the light-brown to dark-brown substances of unknown constitution which are formed in nature by the decomposition of organic matter through the agency of atmospheric agencies or in the laboratory by chemical reagents," and the second as "those humic substances which are capable of giving hydrogen ions and form typical salts with strong bases." The nitrogen was considered as an impurity. No attempt was made at a systematic study of the origin of the soil organic matter, which would throw light upon the nature of these so-called "humic acids."

Just as did the earlier investigators, Oden isolated his "humic acid" from peat soils, by treating them with acid, washing with water, and extracting with four-normal alkali solution. The alkali treatment was repeated 15 to 20 times. The solution was then treated with sufficient NaCl to make two-normal. A coagulum was formed and filtered off; the filtrate was then concentrated and acidified with hydrochloric acid. The precipitate contained both the so-called "humic" and "hymetmelanic" acids, which were separated by alcohol extraction. By means of the potentiometer, Oden demonstrated that the "humic acid" ion is actually present in the soil; he suggested, therefore, that the acid nature of the soil organic matter is due to actual hydrogen ions formed by "humic acid." By determining the hydrogen-ion concentration of this "humic acid" prepared from peat, Oden obtained a reaction of pH 3.87,

but the reaction of natural peat materials was found to range from pH 3.18 to 4.09. Gillespie and Wise (82) found that the hydrogen-ion exponent of "humus," after the first preliminary washings, was pH 4.15; when normal KCl solution was added for washing, the acidity increased to pH 3.3, but on washing further with HCl solution, the exponent increased to pH 4.65. The existence of a soil acidity of pH 4.0 to 4.5 could, therefore, be explained by assuming the actual liberation of free hydrogen ions or the existence of a "humic acid." How could a greater acidity, which can readily be demonstrated in certain peat and other soils, be explained? Oden recognized this fact and suggested that the acidity of acid soils is caused by other substances than those classified as "humic acids." Oden's theory—that "humic acid" is a tetra-basic acid, of a definite chemical composition and free from nitrogen—could also not account for the nitrogen which is usually present in these "acids," in amounts ranging from 2.5 to 3.5 per cent. On repeated solution and reprecipitation, the nitrogen content of the preparation was reduced from 2.5 to 0.7 per cent, thus confirming the results of Detmer (50), who claimed that the nitrogen is an impurity of the "humic acid." This lower nitrogen content could not correspond, however, to the "humic acid" with four replaceable hydrogens. Oden, therefore, also concluded that the nitrogen was probably an impurity. To explain the existence of specific "humic acids," Oden had to suggest that the soil acidity is due not to its hydrogen ions only, and that the nitrogen always found in definite amounts in these "humic acids" is an extraneous constituent.

Van Bemmelen (25) and Baumann (18) give a different interpretation of the nature of the soil organic matter. According to van Bemmelen (26), "humic acids" are not organic acids; they are amorphous and colloidal in nature, having originated from plant substances by various chemical processes, including those of hydrolysis, dehydration, and oxidation, depending on the microorganisms active in the process and on environmental conditions; the amorphous complex was believed to consist largely of carbohydrate and protein decomposition products. On comparing the "humic acids" from peat soils with the extracts from the sphagnum plants which go to make up these soils, Baumann and Gully (19) also concluded that "humic acids" are not acids. The organic material of the soil was looked upon as a colloidal complex with a high power of adsorption. When a salt is added, the base is absorbed by the colloidal complex, setting the acid free; it is this acid which was thought to cause soil acidity. The electrical conductivity of these so-called acids was found to be much less than that of a 0.5 per cent solution of acetic acid and even that was thought to be due to a slight admixture of organic and inorganic acids as impurities.

The rôle of "humic acids" in producing soil acidity is thus explained: 1. by their absorption power, when considered as colloidal complexes (19); 2. by the dissociation of hydrogen ions when considered as organic acids (4, 5, 82, 161, 162) or 3. by the replacement of the inorganic acid, when treated with salts (47, 115).

This brief historical review of the nature of soil organic matter indicates our entire lack of definite systematic knowledge concerning this important soil constituent and the numerous conflicting opinions on the subject. It should be mentioned here, however, that even those investigators, like Oden, who believed that "humic," and other acids are definite chemical compounds, admitted that these are accompanied in the soil by a large number of other chemical substances, which may be classified as follows:

1. Various inorganic and organic acids, such as phosphoric, sulfuric, formic, acetic, propionic, malic, levulinic, oxalic, succinic, dihydroxystearic, and picoline-carboxylic.
2. Aldehydes (214), waxes and fats. Post (175) found that air-dry soil rich in organic matter contained 0.52 per cent fats and waxes; Reinitzer (178) found 0.184 per cent ether extractives in a forest soil, of which 0.154 per cent was wax and 0.03 per cent fat; Fraps and Rather (79) found an average of 0.0203 per cent ether extract and, in addition, 0.0174 per cent

chloroform extract, on an average of 28 Texas soils. According to Schneider and Shellenberg (199, 200), the quality of ether extractives in peat increases with the age of the peat. Dachnowski (46) reports as much as 4 per cent of ether-soluble substances found in certain peat preparations.

3. Various carbohydrates and alcohols, such as pectins, pentosans (68, 147, 206), phytosterol, agrosterol, and cholesterol. Schreiner and Shorey (203) found that the pentosan carbon made up 1.30 to 28.5 per cent of the total carbon of the soil; V. Feilitzen and Tollens (68) even suggested the possibility of using the pentosan content of peat as a measure of the degree of its decomposition [see Gorbenko (83)]. According to Fraps (77), there is a more or less definite ratio between the pentosan and nitrogen content of the soil.

4. Nitrogenous substances, like xanthin, cytosin, histidin, arginin, leucin, isoleucin and other protein-split products, isolated by Schreiner and Shorey (203), Robinson (185, 186, 187), and others. According to Stutzer and Klingenberg (220) some of the nitrogen is present in the soil in a form which is only very slowly available and which consists of plant and animal nucleins, but no attempt has been made to learn whether these substances are present in the soil as such or in the form of more complex compounds. A large part, if not most, of the nitrogenous compounds reported to have been found in the soil are no doubt present as dead and partly decomposed bodies of microorganisms or certain plant residues. Schreiner and Shorey (203), for example, found that dihydroxy-stearic acid occurs in fungus mycelium. Chitin, which is a characteristic ingredient of the cells of various microorganisms has also been often found (155) in the soil, Post-Ramann (175) having recorded that 15 to 20 per cent of the dry weight of peat soils consists of chitin; Höveler (107) could not demonstrate, however, any chitin in peat soils. Potter and Snyder (172) found that the humin nitrogen, as determined by the Van Slyke method of protein hydrolysis, is very high in soils in comparison with the humin nitrogen of proteins; the amount of amino acid and peptide nitrogen found in the soil is very small as compared with the amount of amino acid obtained by acid hydrolysis of soil. Potter and Snyder (172) and Morrow (151) have shown further that the organic matter, as distributed by the Van Slyke method, is essentially the same in soils differently treated and in different soil types. In other words, the nature of the nitrogen distribution in the soil organic complexes is different from that of the nitrogen distribution in proteins of plant and animal origin, a fact also established by Lathrop (129); this nitrogen distribution also indicates a similar kind of nitrogen found in different soils.

#### *Quantitative methods of measuring "humus" in the soil*

Before considering the various theories explaining the origin of "humus" in the soil, it is of interest to review the methods commonly used for determining quantitatively the organic matter content of the soil. Some of these methods are based upon the determination of the total organic matter in the soil, whereas others measure only that part of the organic matter which is more readily oxidized; some determine the organic carbon, others measure, by different methods, the portions of the organic matter which is soluble in alkalies, whereas still others determine only the part of the alkaline extract which is precipitated by hydrochloric acid.

The most common method for determining the "humus" content of the soil has been that of Grandjeau (90) and its various modifications (102, 177, 116). The method consists in extracting the soil first with a dilute solution (about 1 to 10 per cent) of hydrochloric acid, for various lengths of time (until free from calcium), then washing with water. This extraction is presumably for the purpose of washing out the soil bases, with which the "humus" or "humic acids" are otherwise combined. The washed soil is then extracted with ammonium

hydroxide and the clear extract evaporated to dryness, weighed, and ignited to determine the ash content. In other methods, the alkaline extract is precipitated with acid and the precipitate weighed.

It was found, however, that the concentration of the ammonia solution and the time of extraction are of importance; Huston and McBride (108) suggested, therefore, the use of a 4 per cent ammonia solution and a 12-hour extraction period. The ammonia was found to deflocculate the clay, which is not removed by filtration, giving varying results. Various suggestions were then made to remove the clay by filtration through a clay filter (39, 40, 116); to coagulate the clay by evaporation on a water bath (150) by the use of ammonium sulfate (218) or potassium chloride (78); or to separate the clay by electrolysis (177) or by centrifuging (243). None of these modifications, however, made the determinations of "humus" more reliable. The fact that different alkalies extract different amounts of "humus," and that the latter varies in composition, depending upon the nature of the extracting agent (84, 85), served further to discourage the laying of too much emphasis upon the quantitative determination of "humus." Alway, Files, and Pinckney (9) and Fraps (76, 77), recognizing that a large number of the past determinations of "humus" were unreliable, ascribed this to the high ash content.

The "humus" determined by the various methods represents a mixture of organic substances obtained as a result of a certain chemical operation; every modification in the method results in the modification of the nature of the substances extracted. Oden (162), working with peat soils, said, "These methods may be satisfactory for mineral soils, but not for peat soils rich in organic matter." However, the organic matter in mineral soils interacts partly with the inorganic soil constituents, especially aluminum salts; a part of the organic matter forms a complex with inorganic materials, which is soluble both in acids and alkalies, but is precipitated at a definite isoelectric point of pH 4.8 (237). As a result of this, the amount of organic matter extracted will depend on the nature of the reagent used.

Piettre (171) used pyridine for the extraction of "humus" from soil. The "humus" obtained after the solvent is evaporated, is extracted with ether and alcohol and is ashed. The residual soil may be dried, extracted with dilute HCl, washed and dried, then again extracted with pyridine. Balks (17) found that pyridine extracts only the "humified" organic matter, whereas the straw particles remain practically unattacked; but the results thus obtained are claimed to be unreliable.

Colorimetric methods (20, 166, 145, 56) have often been utilized for measuring the "humus" content of the soil or "the degree of humification" of the soil organic matter. A 10 to 15 per cent NaOH solution is used for the extraction of the "humus;" the color of the unknown extract is compared with that of a known solution of "humic acid." According to Oden (162) an increase in the concentration of alkali does not increase the "humification number." Gortner (84) reported, however, that the soil pigment is not necessarily the same substance as the soil organic matter. Alway and Pinckney (11) found that, for surface soils of the same locality, the intensity of color of the ammonia extract is closely concordant with the amount of "humus" present; but, on comparing semi-arid and humid subsoils, they obtained a brown color for the first and no color for the second, although both soils contained the same amount of "humus" when determined gravimetrically.

In many instances, the determination was limited to only one of the constituents of the alkaline extract of soils, such as the methoxyl group (146, 207), total carbon (84, 85, 246), total nitrogen (101, 112), amino or amide nitrogen (119, 52, 13, 14, 123, 223, 172). The results were, however, far from uniform. The discrepancies in the nitrogen determinations of

the soil "humus" are especially illustrative. Hilgard and Jaffa (103) and Loughridge (137) measured the nitrogen content of "humus" by subjecting to the Kjeldahl method the ammoniacal extract of soil, which was evaporated to dryness. A very high nitrogen content running up to 20 per cent was found. Instead of considering the possibility of a faulty method, since no natural organic substance could contain more nitrogen than the purest proteins, they concluded that this high nitrogen content is characteristic of arid soils. On the basis of these faulty determinations, they constructed a theory that although humid soils are rich in "humus" of a low nitrogen content, arid soils contain a smaller amount of "humus" but of a higher nitrogen content. Hilgard saw in this a tendency to equalization of the "total nitrifiable nitrogen content," as the "humus" nitrogen was referred to, due to the fact that it is this form of nitrogen that was supposed to be the most available in the soils of the two regions. This statement was accepted without any attempt at a critical review of the results by the subsequent text writers (247). C. B. Lipman (131, 132), pointed out, however, that the results of Hilgard were due to the faulty method used in extracting the soil "humus." When sodium hydroxide instead of ammonium hydroxide was used for extraction, no difference was found in the nitrogen content of the "humus" of arid and humid soils. This was further substantiated by the results of Sievers and Holz (212), who found that the carbon-nitrogen ratio of soils, irrespective of their origin, is practically constant. A detailed review of the various methods for the determination of "humic acid" in the soil is given by Hoering (104) and Oden (162). White and Holben (246) found that the carbon-nitrogen ratio of the "humus" obtained by washing soil (20 gm.) with 3 per cent NaOH on a Buchner funnel (150 cc. collected) is the same as that of the soil itself. By making the extraction in a shaking machine, the amount of "humus" obtained increased with the length of extraction; shaking for 88 hours with 3 per cent NaOH gave 56.8 per cent of the "humus," whereas extracting for 3 hours on a Buchner funnel gave only 30.5 per cent. By comparing the amount of "humus" in the soil with the crop yield, for a series of differently treated plots, they came to the conclusion that the determination of soil humus, by multiplying the total organic carbon of the soil by 1.724, is a more reliable index of soil productivity than the determination of "humus" by extraction with alkalies.

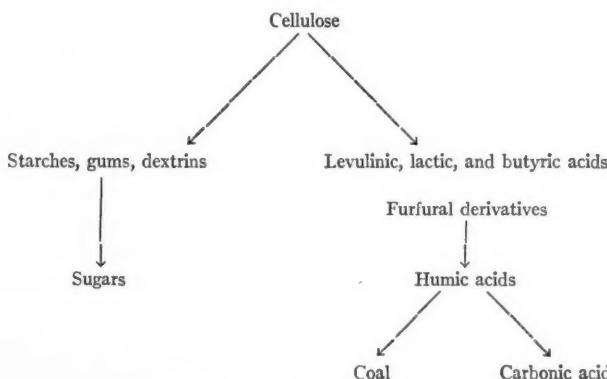
Various other methods are commonly employed for determining the quantity of the total or "available" soil organic matter:

1. The liberation of  $\text{CO}_2$  from carbonates (finely divided  $\text{CaCO}_3$ ) by the free "humic acids" (224). This method, which was later modified by Sückting (221), consisted in treating the "humus" with a definite amount of  $\text{Ba}(\text{OH})_2$  solution, then adding an excess of  $\text{BaCl}_2$  and titrating back the excess of hydroxide with standard acid.
2. The amount of iodine liberated by an equivalent portion of "humic acid" from iodates in the presence of potassium iodide (19).
3. The color of the aqueous solution of "lithium humate" formed by the action of "humic acid" upon  $\text{Li}_3\text{PO}_4$  (4, 5).
4. Titration of soil "humus" with  $\text{CaCl}_2$  solution. The method was found (33) to yield only unreliable results.
5. Oxidation of soil organic matter with  $\text{KMnO}_4$ , followed by determination of the organic carbonate going into solution (16, 109). Fallot (67) found that 8 gm. of oxygen used (as  $\text{KMnO}_4$ ) is equivalent to 3 gm. of carbon or 6 gm. of organic matter.
6. Treatment of 1 or 2 gm. of soil with 60 cc. of 6 per cent  $\text{H}_2\text{O}_2$  solution for 15 minutes, filtering, washing with hot water and determining the loss of organic matter by ignition (121, 188). It is interesting to note here that König and Rump (122) found that lignins could be removed from wood by oxidation with  $\text{H}_2\text{O}_2$ .
7. Oxidation with silver chromate, then multiplying the carbon content by 1.742 to give the quantity of "humus" (17).

*Origin of soil organic matter ("humus" and "humic acids")*

The earlier investigators studying the nature of soil "humus" and the various "humic acids" concerned themselves very little with its origin. Some vague statements that "humus" is a result of decomposition of natural organic substances of plant and animal origin by the action of atmospheric agencies or by microorganisms was sufficient. Baumann (18) and others considered "humic acids" to be a mixture of undecomposed plant tissues, such as the cell membranes of the hyaline sphagnum cells (in the case of peat soils), and partly decomposed substances of plant and animal origin, in the form of a colloidal complex. Hilgard (102) regarded soil "humus" as a definite soil product formed from vegetable material in the soil, under the influence of fungi and bacteria under aerobic conditions; he believed that nitrogen in plant residue must first pass through the "humus" stage before it can be nitrified and become available for the growth of higher plants. An extract similar to "humus" was obtained (78, 86) from fresh plant materials and it was found that when these materials are added to the soil, an actual decrease in "humus" takes place. The idea was then suggested that "humus" is not formed in the soil but is added to it and actually diminishes in the soil. These considerations seem contrary to our ideas of the resistance of soil "humus" to decomposition and of its accumulation in the soil. Some investigators (204, 111, 12) considered "humus" to be a complex of various substances formed from the decomposition of carbohydrates and proteins added to the soil.

Some of the earlier workers, like Detmer (51) and Czapek (45), considered celluloses as the mother substances of "humus," which was looked upon as some intermediary substance between the celluloses and the final product of decomposition, namely carbon dioxide. Among the more recent workers Bergius (28), Jones and Wheeler (114a), Chardet (42) and Marcusson (142, 143) are also of the opinion that celluloses give rise to "humus" or to "humic acids" and to coal. Bacteria are believed to change celluloses into oxycellulose, which is then changed into "humal" acid, then into "humic" acids. The fact that some peats contain a considerable amount of oxycellulose and that the organic complex obtained by the use of a dilute alkaline solution is different from that obtained by the use of a more concentrated solution, also that dark colored substances originate by warming furfural, which may originate from cellulose, was considered as sufficient evidence for this theory. Bottomley (34) and a number of other investigators claim that natural and artificial "humus" are the same. Chardet (42) believed that, under aerobic conditions, the celluloses are decomposed by bacteria into dextrins and sugars, and these into fatty acids and  $\text{CO}_2$ ; and that under anaerobic conditions, as in peat soils, oxidation processes do not take place and "humic acids" may be formed by the processes of condensation, according to the scheme on following page.



Hoppe-Seyler (106) was the first to demonstrate that celluloses and hemicelluloses do not contribute to the formation of "humic acids." He called attention to the rôle that lignin plays in this process. The more or less resistant xylans also remain undecomposed for a considerable time; they are soluble in alkalies and are precipitated by acids, thus forming a part of the "humic complexes." Unfortunately, Hoppe-Seyler carried out his studies with paper immersed in liquid media and inoculated with anaerobic organisms, whereby the growth of fungi and actinomycetes was eliminated. Ehrenberg (58) also expressed doubts as to cellulose being the mother substance of soil "humus," since he could not obtain any "humus" in the decomposition of cellulose or its derivatives at normal soil temperatures. Snyder (215) was the first to study "humus" formation under conditions which would correspond to normal soils. "Humus" was formed at the expense of sugar, oat straw, clover hay, etc.; no attempt was made, however, to determine whether this "humus" originated from the organic materials themselves or was merely a result of the development of fungi. Trusov (231) actually demonstrated that there is no direct formation of "humus" from celluloses; indirectly these substances may have an important bearing upon the process, through the synthesis of microbial cell substance. This is true of course of other carbohydrates. Various other investigators (164, 223, 27) did not consider celluloses as "humus"-forming materials. According to Benni (27), the proteins of plant and animal origin, certain carbohydrates (not celluloses), and a few plant acids contribute to the process of "humus" formation: the oxidation of the proteins and their derivatives is supposed to give a nitrogen-containing "humic acid" and the oxidation of the carbohydrates and organic acids yields a nitrogen-free "humic acid," a mixture of the two being "humus."

The positive rôle of proteins in the formation of "humic acids" has been suggested by the work of various investigators; this process which takes place in the soil has been frequently compared to the formation of the dark colored melanins on boiling proteins with acids or alkalies. The subject has resulted in

an extensive literature (43, 193). However, Mulder (154) and Schmiedeberg (198) emphasized the fact that no two "humus" bodies ever obtained agreed in their composition. The formation of the dark colored substances in the boiling of proteins has been ascribed (128) to the carbohydrate present in the protein molecule or to certain specific amino acids, such as tryptophane or tyrosine (165, 87, 88). The benzol and pyrrhol nuclei are largely concerned with the formation of "humus," which results from the activities of micro-organisms. It is sufficient to point to the formation of homogentisic acid from tyrosin by the action of actinomycetes. The same is true of other products of protein decomposition, such as p-cresol, hydro-p-cumaric acid, and other phenol derivatives.

The protein nature of soil "humus" was first suggested by G. Fischer (72). Suzuki (223) and Jodidi (111) came to the conclusion that "humic acids" contain an insoluble body, in the nature of a protein, which contains the nitrogen of the organic matter. In this they confirmed the previous observations of Berthelot and André (29, 30), Sestini (207), Dojarenko (52), and Baumann (18), who found the soil nitrogen to be in the form of amino- and amide compounds, and of Sievers (213) and Grouven (93), who considered the nitrogen to be present in the soil "humus" in the form of a protein. Detmer (50) was the first, however, to demonstrate that nitrogen exists in the soil in the form of organic compounds. But Detmer himself and later Sestini (207), Oden (162), and Eller (64) were led to believe that the nitrogen is only an impurity of the "humus" or "humic acids" found in the soil, since on boiling these with hydrochloric acid, the nitrogen can be reduced from the 2.5-3.5 per cent to 0.7-1.5 per cent. One might be more justified in concluding that boiling soil organic matter with hydrochloric acid hydrolyzes the nitrogenous part of this organic complex more readily than the non-nitrogenous portion. Eggertz (57) found that, on reprecipitation, "humus" may become richer in nitrogen.

One may dispose easily of the earlier ideas of Mulder (154), Ritthausen (183) and others that the "humic acids" absorb the nitrogen only in the form of ammonia. The nitrogen found in the soil organic matter may be looked upon as either forming an integral part of the soil "humus" or as being present there in the form of proteins, as suggested by Berthelot and André (29), Eggertz (57), Shmook (208) and others. These proteins probably form definite complexes with the other constituents of the soil "humus." Whichever theory is accepted as the correct one, one fact is certain, that the nitrogen of the soil organic matter is derived either from the nitrogenous constituents of the plant materials added to the soil or from the constituents of the various microbial bodies bringing about the decomposition of these materials. It is a well known fact that when proteins and their derivatives are added to the soil, they are rapidly acted upon by the different microorganisms, with the formation of ammonia and other protein derivatives. If these were the forms of nitrogen present in the soil "humus," they could easily be isolated from the

soil without having to subject it to the vigorous treatment with strong acids. Only a part of the nitrogen can ever be found in this form, as shown by numerous investigators (129). Sächtung and associates (222) suggested that the major part of the nitrogen is present in the soil in the form of heterocyclic compounds, such as derivatives of pyridine and its higher homologues, which cannot be decomposed readily by microorganisms; this nitrogen may be made available to plants, either in a normal condition by the interaction of mycorrhiza fungi, or when the soil is heavily limed and aerated. The actual isolation of a pyridine derivative—picoline carboxylic acid—from the soil was accomplished in 1906 by Shorey (209). Most of the studies on the nature of the nitrogen compounds of the soil, however, were limited to the demonstration of various amino acids and amides among the hydrolytic products obtained by the action of strong mineral acids upon the soil organic matter.

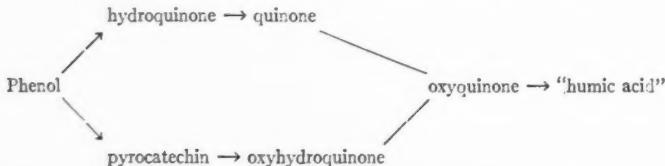
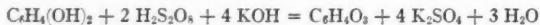
Lathrop (129) demonstrated that the decomposition of proteins in the soil is accompanied by various synthetic processes, including the formation of new proteins and nucleic acids which are more resistant to decomposition. The new protein is characterized by a much higher amide content than vegetable and animal proteins and points to the presence of considerable amounts of microbial protein in the soil.

Nägeli (157) was the first to ascribe to filamentous fungi the main rôle in the formation of crude "humus." Kostytscheff (124) demonstrated that "humus" is formed from various organic substances when acted upon by fungi and not by bacteria. Müller (155) considered *Cladosporium* as an important organism taking an active part in this process; König (120) ascribed an active rôle in humus formation to *Trichoderma koningi* and *Cephalosporium koningi*; Höveler (107) considered fungi as the most important agents in the process of "humification;" the results of Scherpe (196) point also in the same direction. According to Beijerinck (23), lignin is changed to "humus" chemically, whereas the epidermis and the dark tissues are acted upon by fungi. Beijerinck considered the actinomycetes, because of their ability to form quinone from proteins formed in the cells of the organisms, to be active in the process of "humus" formation. The fact that fungus mycelium penetrates woody tissues and organic particles of forest and peat soils has been recorded by various investigators (180, 176). According to Frank (75), these fungi make up a considerable part of the soil organic matter and the nitrogen of the "humus" is largely derived from the cells of microorganisms. The formation of pigments by various bacteria (233) may play a part in the formation of the dark colored substances referred to as "humus." Beijerinck (24) and Rippel and Ludwig (181) found that the dark pigment of *Azotobacter chroococcum* is in the nature of melanin which is formed from tyrosin; it dissolves in sodium hydroxide solution.

It seems to be generally agreed (134) that under anaerobic conditions, celluloses are completely decomposed to carbon dioxide, methane, and fatty acids, whereas, under aerobic conditions, various dark colored substances are left by

the bacteria and fungi. These substances are constituents of the microbial protoplasm and are not secretion products or intermediary products formed from the decomposition of natural organic substances. This has been demonstrated, in the case of fungi, by Daszewska (48), Traaen (230) and Waksman and Heukelekian (238); the cellulose is decomposed completely and all its carbon can be accounted for by the carbon dioxide of the atmosphere and the carbon assimilated by the microorganism. The only residual substances are the microbial cells. Our present information concerning the origin of "humus" or "humic acids;" namely, the dark colored organic substances which resist further rapid decomposition can be summarized as follows:

1. *The formation of "humus" as a result of oxidation of benzene-ring compounds.* Hoppe-Seyler (106) and Reinitzer (178) demonstrated that dark colored substances ("humus") are formed on boiling phenols and quinones with alkalies. According to Eller (64), the oxidation of phenol, quinone and hydroquinone in an alkaline solution results in the formation of acids similar, not only in composition (58.05 per cent carbon) but also in the various physical and chemical reactions, to natural "humic acids," of the formula  $x$  ( $C_6H_4O_2$ )



The natural "humic acid" obtained from brown coal with sodium hydroxide solution and precipitated with an acid was found to contain 59.6 to 60.2 per cent carbon, 3.2 to 3.4 per cent hydrogen, 1.7 to 2.0 per cent nitrogen, 1.2 to 1.9 per cent sulfur and 1.4 to 2.4 per cent ash. The nitrogen was looked upon as an impurity and not as a normal constituent of the "humic acid." Eller believed that natural "humic acids" originate by the oxidation of hexoses, as



He thus agreed with Bottomley (33), Mascusson (143), Jonas (114) and Jones and Wheeler, (114a) who ascribed the furan structure not only to the synthetic "humic acids" from carbohydrates, but also to the natural "humic acids." Muschel (156) found that the black coloration of the nutrient media of *Bac. mesentericus niger* was due to components of the benzene ring series, closely related to o-and p-dihydroxybenzene, which apparently form condensation products with amino acids. Similar results were obtained by Perrier (169), who found that when alkali salts of benzoic and salicylic acids and phenol are acted upon under aerobic conditions by fungi and bacteria (*Bact. pyocyaneum*) a characteristic dark colored substance is formed. Perrier concluded that the dark colored substances of "humus" are formed by the oxidation, at an alkaline reaction, of cyclic compounds present in animal excreta and in vegetable substances, after they have decomposed. The formation of dark colored substances from tyrosin and other phenol derivatives by actinomycetes was referred to previously.

2. *The formation of "humus" is believed to be a result of the interaction of carbohydrates with amino acids or polypeptides.* Various investigators recognized that nitrogen is an essential part of soil "humus." André (13) claimed that this nitrogen is fixed so energetically to the carbon of the "humus" that when it is boiled with a solution of NaOH or KOH, only a part is

eliminated as  $\text{NH}_3$ ; only prolonged treatment will remove more of it. This confirmed the earlier observations of Eggertz (57). Maillard (140) considered that nitrogen plays a necessary part in determining the natural processes of humification.

As far back as 1861 Thénard (227) stated that "humic acid" found in manure is not derived from animal material but by the action of animal matter upon lignins or substances extractable from straw or wood. By extracting manure with warm water and precipitating the alkaline liquid with hydrochloric acid, Dehérain (50) obtained a product which analyzed 59.2 to 63.0 per cent carbon and 3.0 to 4.7 per cent nitrogen. Dehérain (49) as well as Hébert (94) observed that, in the rotting of manure, a constant transformation of the ammonia nitrogen into organic nitrogen takes place. Dehérain recognized in it the formation of microbial protoplasm. Maillard (139) believed this to be a purely chemical reaction of sugars and amino bodies. He believed that the rôle of microorganisms in this process consists in the breaking down of proteins to polypeptides and amino acids; the actual formation of "humus" is, according to Maillard, an automatic chemical reaction (between the amino acids and carbohydrates), in which microorganisms play no part. The natural and artificial humic substances (latter obtained by condensation of sugars with amino acids) were believed identical in composition, resistance, and nitrogen [see also Roxas (191)]. However, Neuberg and Kobel (158) could not obtain either melanin or  $\text{CO}_2$  formation as a result of interaction of fructose with alanin in the cold, even after weeks. Although dark colored bodies are formed in the interaction of amino acids with sugars at high temperatures and in concentrated solutions, it is doubtful whether this would take place to any extent in the soil (110, 231).

3. "*Humus*" is formed from the polymerization of furfural. When furfural is boiled with hydrochloric acid, it is converted into a black insoluble mass (87, 88, 142). It was suggested, therefore, that "humin," which is formed when carbohydrates are boiled with acids, is actually formed from furfural, which is produced in its turn from carbohydrates. According to Beckley (22), hydroxy-methyl furfural is formed by the action of mineral acids on carbohydrates, and, on condensation, that compound yields "humus;" hydroxy-methyl furfural was also demonstrated in the rotting of straw and in the soil, but not in the decomposition of cellulose by pure cultures of bacteria.

4. *The formation of "humus" from lignins.* The probable rôle of incrusting substances in the formation of "humus" in the soil has been suggested by various investigators during the last decades of last century; some even considered lignins as the chief sources of "humus" (106). Lange (127) and Schwalbe (205) also expressed the idea that humic acids are closely related to the lignic acids. According to Hofmeister (105) dark colored substances are formed by the extraction of lignin with alkali, giving finally "humic acids." Dehérain (49) records the results of Hébert who found that when natural organic matter is added to the soil, the sugars and dextrins disappear rapidly and completely; the celluloses and hemicelluloses diminish appreciably whereas the lignins are attacked least. Dehérain adds that the lignin is soluble in alkalies and forms the "matière noire." The nitrogen part of the black material ("humus") is derived from ammonia which is assimilated by microorganisms for the synthesis of proteins, the available carbohydrates being used as a source of energy. "Humus" thus represents a mixture of lignins and proteins. Such a clear definition of the problem of "humus" formation has been altogether overlooked by subsequent investigators on the subject.

Wehmer (240, 241) found that, in the degradation of wood by fungi, especially certain higher fungi, the lignocelluloses are attacked, the celluloses disappearing and the lignins becoming converted into "humic substances." He suggested that, in peat and coal formation, the plant tissues are first converted into "humic" substances by the fungi and not by the bacteria; the lignins, however, cannot be attacked by fungi. According to Trussov (231), celluloses, hemicelluloses, lower saccharides, and glucosides take no part in the formation of "humus;" lignins, proteins, pigments and tannins give rise to the typical black "humus." Fats and waxes do not change into "humus," but because of their slow decomposition, they form a

part of it. The results of Bray and Andrews (37) and of Du Toit (54) on the decomposition of wood by different fungi tend to demonstrate further that when organic matter is acted upon by microorganisms, the celluloses are rapidly decomposed, the pentosans may remain partly undecomposed, and the lignins remain intact or are acted upon only to a very limited extent.

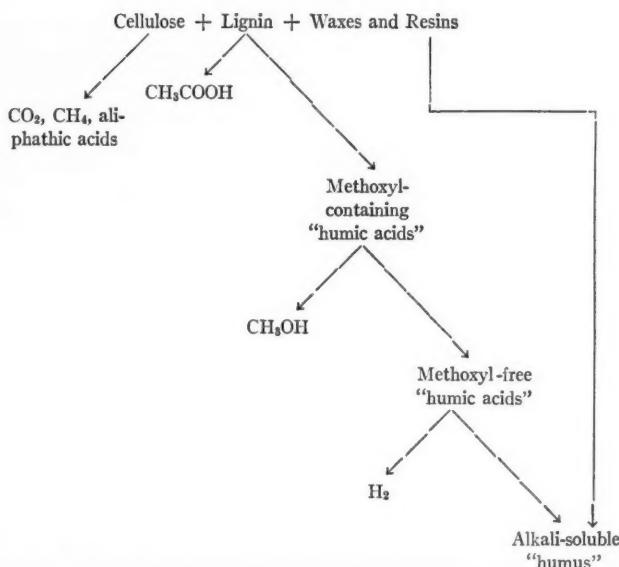
The most extensive work on this subject is that of Fisher and his associates (70, 71), who submitted a very interesting thesis concerning the important rôle that lignins play in the formation of "humus." They have pointed out that cellulose has an aliphatic or furan-like structure, but that lignin has an aromatic structure containing the benzol ring with acetyl and methoxyl groups. When plant substances are decomposed the celluloses change to  $\text{CO}_2$  and water, whereas the lignin remains and accumulates in peat materials; as a result of the breaking off of the acetyl group, the phenol-containing alkali-soluble body of lignin is changed to "humic acids." By an increase in the size of the molecule, either by oxidation or dehydration, the "humic acid" is changed into insoluble "humin," and then into coal. This theory was substantiated by the following considerations:

	 (FURAN-DERIVATIVES)	 (BENZOL-DERIVATIVES)
Cellulose.....	+	-
Lignin.....	-	+
Sugar.....	+	-
Artificial "humus" from sugar.....	+	+
Natural "humus".....	-	+
Coal.....	-	+

By inoculating with a soil suspension cellulose, lignin, wood, and sphagnum moss, placed in an inorganic nutrient solution and incubating at  $37^\circ$ , fungus development took place in the moss, and later in the wood and cellulose but not in the lignin prepared by Willstätter's method. These and other considerations led Fisher (69) to conclude that lignin is the mother substance of natural "humic acids." Fischer suggested that when natural organic substances are decomposed (under conditions of peat formation), the processes on following page take place.

Under favorable conditions, the "humus" loses water, methane, and carbon dioxide, giving bituminous coal together with the resins and waxes.

A decrease in the cellulose content of peat during decomposition and with age of material has been observed by v. Feilitzen and Tollens (68) and by Keppler (117), and a considerable increase in the proportion of the organic material soluble in alkalies, referred to as "humic acids," in the older layers of peat has been recorded by Schneider and Schellenberg (200). Thus the formation of peat can be looked upon [Thiessen (228)] as the decomposition of natural organic matter under anaerobic or water-logged conditions, whereby the soluble carbohydrates, hemicelluloses, and celluloses are decomposed, while the lignins and the protective substances (waxes, etc.) accumulate. Under those conditions, the fungi are eliminated while the cellulose-decomposing bacteria are found in abundance. The type of peat formed will depend on the completeness with which the celluloses are disintegrated; most peats (fibrous peats) still contain some undecomposed celluloses. "Humus" compounds obtained from peat can be separated, on the basis of their chlorine derivatives, into two different types, one of which is similar to a lignin compound and one to "artificial humus" obtained by the action of mineral acids on carbohydrates or from cellulose fibres decomposed through aging. It has been suggested that the second compound present in peat is due to decomposition of cellulose by chemical agencies (225). Assuming that the silica content of saw grass



was not diminished during peat formation, Miller (148) calculated that seven parts of the saw grass were required to yield one gram of peat, whereby 33 per cent of the original nitrogen was lost. Other recent contributions (170, 189) tend to substantiate the theory concerning the rôle of lignin in peat and coal formation. Rose and Lisse (189) found that the decomposition of wood goes hand in hand with a decrease in the cellulose and an increase in the lignin content, as determined by the methoxyl ( $\text{CH}_3\text{O}$ ) groups characteristic of lignin [Zeisel method (251)] and of alkali-soluble material:

WOOD	CELLULOSE per cent	METHOXYL GROUPS per cent	ALKALI-SOLUBLE MATERIAL per cent
Fresh.....	59.0	3.9	10.6
Half decomposed.....	41.7	5.2	38.1
Completely decomposed.....	8.7	8.7	65.3

Similar results were obtained by Balks (17), by Fischer, Schrader, and Friedrich (71) and by König (120a). The presence of methoxyl in the organic matter of mineral soils has been demonstrated by Shorey and Lathrop (210). The results obtained by Dore and Miller (53) on the decomposition of wood by *Teredo* are of interest in this connection; a chemical analysis of the wood borings ejected by *Teredo* and of the original wood from which the borings were derived gave the following results:

	SERIES I			SERIES II	
	Wood (parts per 100)	Borings 1 (parts per 56.1)	Borings 2 (parts per 54.7)	Wood (parts per 100)	Borings (parts per 50.9)
Hemicellulose.....	6.02	3.62	5.10	14.23	6.20
Cellulose.....	57.74	11.79	10.99	47.45	10.96
Lignin.....	30.60	30.60	30.60	27.84	27.84
Furfural yield.....	5.37	3.32	3.32	5.90	4.26

These results indicate definitely that the *Teredo* utilizes readily the celluloses and hemicelluloses, but not the lignins. König (120a) has shown that in the digestion of plant tissues by herbivorous animals, the celluloses are attacked much more rapidly than the lignins.

None of these four theories fully explains the nature of soil organic matter commonly referred to as "humus" or even the part soluble in alkalies and precipitated by hydrochloric acid, referred to as "humic acids." The first three theories assume the existence of strong mineral acids, soluble carbohydrates, or free amino acids. None of these can exist in the soil for a very long time. Mineral acids would not be produced in more than mere traces in any normal soil, and will then immediately be converted into salts by the soil bases. Soluble carbohydrates will be attacked immediately in the soil by the numerous fungi and bacteria in the presence of available nitrogen or by nitrogen-fixing bacteria, in its absence. Free amino acids will be acted upon immediately by the numerous soil organisms which will utilize them either as sources of nitrogen, energy, or both, or for the structure of their protoplasm. The formation of mere traces of "humus" is possible by these processes. The fourth theory has sufficient basis to deserve careful study and consideration. The similarity in physical and certain chemical properties, of the lignins and the "humic acids" and the fact that neither are readily acted upon by microorganisms give weight to this theory. Both lignins and "humic acids" are soluble in alkalies and precipitated by hydrochloric acid, both contain methoxyl groups, both darken on oxidation, both are acted upon by  $H_2O_2$ , and both absorb water. The theory that lignins are the mother substances of soil "humus" fails, however, to account for one very important constituent of this humus, namely the nitrogen. Lignin is free from nitrogen, whereas the "humic acids" prepared from peat and mineral soils always contain 2 to 4 per cent of nitrogen (usually 3 per cent). Moreover, the existence of a constant ratio between the carbon and nitrogen content of the soil has been definitely established. It is true that this nitrogen is unevenly distributed among the soil fractions separated by alkali treatment, as shown by Berthelot and André (29), but the ratio between the carbon and nitrogen seems to be balanced for the soil as a whole, ranging usually from 12:1 to 8:1. It is not sufficient and hardly scientific to explain this phenomenon by the fact that nitrogen is present as an impurity of the "humic acids" in the soil, as was done by Detmer (50), Eller (64, 65) and Oden (162), simply because the nitrogen content did not fit into the particular formula for the supposedly pure "humic acid." It is true that the soil nitrogen is present in a complex organic form, although probably not as free protein, otherwise it would be rapidly decomposed by the soil microorganisms. These nitrogen complexes (222) either are not readily acted upon by microorganisms or only by certain specific forms that develop only under certain conditions, or they are closely connected with a non-nitrogen group of a high carbon content, the complex as a whole not being attacked by the great majority of soil microorganisms. Once the freshly added organic matter has decomposed rapidly, the remainder decomposes rather slowly. The nitrogen is

liberated as ammonia and then oxidized to nitrate, while the carbon is liberated as  $\text{CO}_2$ , the ratio between the combined carbon and nitrogen in the soil remaining always constant, at about 10-1. Eller and Oden reduced somewhat the nitrogen content of their humic acids only after repeated treatments with strong alkali. This may indicate that the nitrogen-bearing fractions of the soil organic matter can be to some extent more readily hydrolyzed than the bulk of the non-nitrogen bearing fraction. As a matter of fact, Berthelot and André (30) and others also found that the treatment of the soil with alkalies brings into solution a proportionately much greater amount of nitrogen to carbon than is left in the soil or than can be precipitated out by hydrochloric acid. But this is hardly sufficient reason to assume that definite chemical compounds exist in the soil as "humic acids" which are free from nitrogen, and that the nitrogen is present only as an impurity. The lignin theory does not account for this nitrogen and its relation to the other soil organic constituents.

It is also important to note, in this connection, that "humus" actually may accumulate in the soil, but, when a soil is properly limed and well aerated, the "humus" disintegrates and gradually disappears. This indicates that a certain set of conditions, as in the case of peat soils, favors the accumulation of humus, whereas another set of conditions, as found in normal cultivated soils, favors the slow gradual decomposition of the "humus." Gehring (81) found, for example, that when soil is fallowed the total organic matter is somewhat reduced, in comparison with the same soil receiving stable manure or clover. This is accompanied by a very considerable reduction of that part of the organic matter which can be oxidized by hydrogen peroxide. This points to the existence of organisms, which, under definite conditions, are capable of decomposing the "humus." These discrepancies can be explained on the basis of the fifth theory concerning the origin of "humus."

*5. The formation of soil "humus" as a result of the synthesizing activities of microorganisms.* According to Post-Ramann (175) and Müller (155), the "humus" bodies obtained from soil often consist of chitinous remains of insects and animal excreta. Kostytscheff (124) and Ollech (164) suggested that these "humus" bodies may often be the remains of bacteria and fungi. Wettstein (244) and Winterstein (249) demonstrated that chitin is characteristic of various fungi and not of bacteria. Schmook (208) advanced the idea that protein nitrogen in the soil was largely present in the bodies of bacteria and protozoa. Trussov (231) also demonstrated that the protoplasm of fungi and probably also of bacteria serves as a source of "humus" in the soil; according to this investigator, all organic substances can thus become indirectly sources of "humus" in the soil, after passing through the bodies of the microorganisms. "Humic acid" preparations often contain large amounts of waxes and resins (195); these may come partly from the natural organic matter and partly from the bodies of microorganisms. Schreiner and Shorey (204) suggested that various chemical substances found to be characteristic constituents of the soil are probably synthesized by microorganisms. These facts are supplemented by other information submitted by various investigators (120, 23, 241, 95) concerning the rôle of microorganisms in the formation of soil organic matter or soil "humus."

Falck (66) differentiated several processes of transformation of organic matter in forest soils, leading to the formation of different types of "humus": (a) The complete decomposition of organic matter by fungi (*mycocriny*), whereby the yearly addition of raw materials is

balanced by the amount of decomposition taking place, without any increase in "humus" content. Fungus protoplasm is hereby synthesized; this serves as excellent fertilizer for the forest trees. The celluloses are decomposed completely, whereas the lignins are more resistant; in some cases (as corrosion by Basidiomycetes) however, the lignins and cellulose are both completely decomposed. (b) Decomposition of organic matter is begun by fungi and then interrupted by lower invertebrates and bacteria (*anthracin*). The fungus mycelium and the original organic matter is devoured by larvae of various insects and worms, producing a dark "humus" mass which is then attacked by bacteria in the presence of basic materials, the action resulting in the liberation of carbon as  $\text{CO}_2$  and nitrogen as ammonia then nitrate. These processes result in the formation of a muck soil. (c) The process of peat formation (*anthrogeny*) is less clearly understood. Falck explains this process by the absence of abundant fungus development.

The author (236) called attention to the similarity between the carbon and nitrogen ratio in the soil organic matter and in the protoplasm of soil microorganisms (largely fungi) and suggested that these probably make up a large part of the soil "humus." When cellulose is added to the soil, it decomposes only in proportion to the available nitrogen contained in the soil liberated by it in a certain period of time (99, 238). This is because cellulose is decomposed in normal soils largely by fungi and also by bacteria, both of which require combined nitrogen. The ratio between the amount of cellulose decomposed and the nitrogen required will be about 30 to 1; i.e., for every 30 parts of cellulose decomposed by the fungi or bacteria, one part of nitrogen will be changed from an inorganic form, like ammonium salt or nitrate, to microbial protoplasm. In the presence of sufficient nitrogen, the decomposition of cellulose by pure cultures of aerobic microorganisms takes place very rapidly. The same is true not only of cellulose but of straw, wood products, corn stover, and other natural substances rich in celluloses, pentosans, and lower carbohydrates and poor in nitrogen. This phenomenon accounts largely for the injurious effect of straw on plant growth. This large amount of nitrogen is needed because above 30 per cent of the carbon of the cellulose which has been decomposed may be changed into carbon of microbial protoplasm. This involves the synthesis of considerable amounts of protoplasm, which results in the storing of large quantities of nitrogen. The constant synthesis of proteins and other complex organic nitrogenous substances by microorganisms in the soil, whether carbohydrates or proteins are used as sources of energy, has been recorded by Dehérain (49), Lathrop (129) and others, as noted above.

While the lignins may contribute the bulk of the soil organic matter, the microorganisms through their synthesizing activities may contribute the nitrogen part of this organic matter

#### *Decomposition of "humus" by microorganisms*

If little is known concerning the nature of the soil organic matter, if the origin of these dark colored organic substances in the soil is little understood, the amount of knowledge concerning the decomposition of this "humus" either by microorganisms or by chemical agencies is certainly very limited. Conflicting statements, vague generalizations not based upon any experimental data, a number of speculations, are all that can be found in the voluminous literature on the transformation of organic matter in the soil. On the one hand statements are made (100, 101, 102) that all organic matter must pass through the "humus" stage before its nitrogen can become available to higher plants; also that soil "humus" can even be used as a source of energy for nitrogen-fixing bacteria (135, 133). On the other hand, the few data obtained by careful experimentation (159, 178, 120) indicate that "humus" is a highly resistant substance and cannot be readily acted upon by microorganisms.

Hoppe-Seyler (106) stated as far back as 1889 that, although "humic acids" afford a habitat to various animals, fungi, algae, and bacteria, no plant or animal is capable of obtaining nutrition from them and no bacterium is capable of decomposing them. Schmidt (197) found that pure "humic acid" cannot serve as a source of energy for Azotobacter or other bacteria. Sphagnum moss and young sphagnum peat can serve as an indirect source of energy, because of the presence of carbohydrates which can be hydrolyzed with dilute acids.

A careful study on the utilization of "humic acids" by fungi was made by Reinitzer (178). The "humic acid" was obtained by treating the soil with a dilute ammonia solution at 30 to 40°C. The liquid was removed and concentrated on a water bath. The residue was again extracted and the filtrate added to the first extract. The concentrated solution was precipitated with hydrochloric acid. The precipitate was redissolved in ammonia and the excess of the latter was removed by concentrating the solution on a water bath. This deep brown solution or the hydrochloric acid precipitate was used as a source of "humus;" it contained nitrogen, potassium, phosphorus, magnesium, and sulfur, sufficient for the growth of the organisms. *Pen. crustaceum* Fries readily developed upon these humus preparations. Reinitzer recognized quite correctly, however, that the methods employed for the extraction of the "humus" from the soil resulted also in bringing into the solution various carbohydrates, such as pentosans, hemicelluloses, pectins, and gums, even if chitin, cellulose, fats, and waxes were not extracted by the dilute ammonia. These carbohydrates, which were also precipitated by the acid, probably served as a substrate for the growth of the fungus. To avoid this, the "humus" preparation was boiled for one or two hours with 5 per cent HCl to hydrolyze the carbohydrates; the residue was washed, redissolved in ammonia, and the excess of the latter removed on a water bath. No fungus development took place on the "humus" so treated, whether in solution or in a precipitated form. On repeating these experiments with decayed wood, exactly similar results were obtained, the crude preparations (lignin in case of wood, "humus" in case of soil) gave some growth of fungi, but when treated with 5 per cent HCl no fungus development took place. The "humus" could be used, however, as a source of nitrogen by microorganisms.

Nikitinsky (159) treated soil with 10 per cent HCl, then washed the soil with water and extracted it with 10 per cent NaOH; the extract was precipitated with HCl, giving "humic acid;" this was washed with HCl and water and dried. It contained 3.22 per cent nitrogen, 4.92 per cent ash, and 51.56 per cent carbon. Some of the "humic acid" was dissolved in ammonia and the "humate" dried on a water bath. Four grams of "ammonium humate" was added to 400 gm. quartz sand to which also 60 cc. of a solution containing 0.2 per cent  $\text{KH}_2\text{PO}_4$  and 0.2 per cent  $\text{MgSO}_4$  was added. The flasks were sterilized, inoculated with soil suspension, and connected with a respiration apparatus. Only about 4 per cent of the carbon was changed to  $\text{CO}_2$  in 9 days by certain aerobic organisms. Nikitinsky (159) came to the conclusion that

humates cannot be used as sources of carbon by microorganisms but can be used as sources of nitrogen.

Robertson, Irvine, and Dobson (184) prepared natural "humic acids" from peat, by extraction with 5 per cent NaOH, then precipitating with excess of strong acid. The product thus obtained contained 57.14 per cent carbon and 2.79 per cent nitrogen. Microorganisms made only a small amount of growth when this preparation was used as a source of carbon and  $\text{KNO}_3$  as a source of nitrogen, and a much better growth with peptone as a source of nitrogen. Robertson and associates suggested, therefore, that their results were contrary to those of Nikitinsky. The former investigators, however, used crude "humic acid," which was found by Reinitzer to allow some growth of fungi because of the presence of impurities. When peptone was added, the improved growth was no doubt due to the utilization of the peptone and not of the "humic acids" as a source of energy.

There is no doubt, however, that there are organisms in nature which are capable of decomposing even purified "humus" preparations, otherwise "humus" would constantly accumulate in the soil; it is known that well aerated and limed soils readily loose their organic matter. Continuous cropping of land, especially with summer fallowing was found to lead to a decrease in the "humus" content of the soil, as shown by Snyder (215), Alway (6, 7, 8, 9, 10, 11, 12), Ladd (126), and numerous others. Rosenberg (190) considered certain cocci to be capable of decomposing "humus." Salzmann (192) and Störmer (219) found that actinomyces can obtain nourishment from peat extracts and from raw "humus." According to Nikitinsky (159), various cocci, rods, and actinomyces are capable of decomposing "humus," but not under anaerobic conditions. Chitin, which was found to be a common constituent in the soil and in the cells of certain soil organisms can also be decomposed by certain bacteria and actinomyces. Winogradsky (248) recently demonstrated that when "humus" extract is added to a silica gel plate and inoculated with soil, certain bacteria ("autochthonous" organisms) develop out of the soil, using the "humus" as a source of energy and nitrogen.

Agafonoff (3) pointed out that, with a definite set of conditions, a definite equilibrium is established between the accumulation of "humus" in the soil and its decomposition. White and Holben (246) found that when soil is treated with lime and manure for a period of 40 years, 90 per cent of the organic matter is decomposed as compared with 84 per cent decomposition of the organic matter added to the soil in the form of manure but without lime. The manured soil treated with lime contained less readily soluble humus than the unlimed manured soil. It is also commonly reported that fallowing of soil results in a diminution in the amount of available "humus."

According to Ehrenberg and Bahr (60, 61), forest "humus" without lime, has an injurious effect upon the growth of economically important crops. Sückting and associates (222) found, however, that forest humus does not prevent the assimilation of added available nitrogen compounds by higher

plants. In 2 years, 3.25 per cent of the nitrogen of forest "humus" was made available when treated with lime. In ordinary forest soils, the nitrogen is probably made available to the trees by the interaction of the mycorrhiza fungi (144). It is sufficient to point also to the common practice of draining and liming peat soils to bring about the liberation of the nutrients. A change from anaerobic to aerobic conditions and from an acid to a less acid reaction of the soil brings about the development of organisms capable of decomposing the organic matter of peat.

The stimulating influence of "humus" upon the activities of microorganisms has been studied in connection with the growth of Azotobacter and nitrogen-fixation. Krzemieniewski (125) first observed that crude "humus" stimulated this process. Fischer (73) recorded the same stimulating influence upon other bacteriological processes and ascribed this to the action of "humus" as an oxygen carrier. Löhnis and Green (135) ascribed the stimulating action to the chemical improvement of the medium. "Humus" was also reported to exert a favorable influence on nitrification (179); this influence was believed to be due to the iron content of the "humus." In view of the fact that no differentiation has been made between the total soil organic matter and the alkali soluble fraction, it is impossible to point definitely to any specific substance, to which the favorable effect upon the growth of microorganisms is due. Further information on the decomposition of forest "humus" by microorganisms is given by Hesselmann (97) and by Möller and Hausendorf (149).

#### *The rôle of "humus" in soil processes*

The important rôle of soil organic matter (or "humus") in soil fertility is well recognized. It is sufficient to mention:

1. The physical properties of the soil organic matter in influencing tilth, moisture, temperature, and the nature of the soil solution.

2. Its chemical properties of combining with soil bases, interacting with various salts. It exerts thus an important influence upon the reaction of the soil, either acting directly as a weak organic acid or by combining with bases liberating the more highly dissociating inorganic acids (235). According to the Daikuvara (47) and Kappen (115), the soil acidity is a result either of the interaction between neutral salt solution and soil "humus," due to the liberation of inorganic acid:



or between aluminum and iron humates with neutral salts, by a process or base exchange. The effect of "humus" in the weathering of silicates is also important in this connection (160). The presence of a considerable amount of nitrogen and minerals, necessary for the growth of higher plants in this organic complex makes it economically of the greatest importance.

3. The biological properties of "humus" in offering a habitat and a source of energy, nitrogen and minerals for various microorganisms (15).

The ability of soil "humus" to absorb bases was first studied in detail by Eichhorn (62). The influence of this phenomenon upon the solubility of phosphates has also been pointed out by Fleischer (74), although Sprengel (217) was the first to demonstrate that phosphates are decomposed by natural

"humic acids." Different phosphates are decomposed to a different extent, different soils and peats producing a different action. The addition of certain salts like KCl and especially  $K_2SO_4$ , stimulates this process, whereas others, like gypsum, injure it. When prepared "humus" is brought in contact with insoluble phosphates, a large part of the latter is changed to monocalcium phosphate and sometimes phosphoric acid (19). As much as 0.56 to 0.98 gm. of phosphoric acid was set free from 15.7 gm. calcium phosphate, when the latter is mixed with 100 gm. of peat. The greater the amount of water available for a given amount of peat and phosphate, the greater is the amount of phosphoric acid brought into solution. This solubilization of the phosphate is due to the adsorption of the base and the liberation of the acid. Sphagnum itself acts as an acid, absorbing calcium from  $CaCO_3$  (123).

It has been shown repeatedly (1), that the acid reaction of sandy and peat soils is caused by "humic acids." The assumption is thereby made that this "humic acid" of both soil types is the same, has the same equivalent weight and probably the same chemical composition. According to Oden (162, 163) the favorable effect of liming acid and bog soils is due to the neutralization of the toxic acids and the formation of "humates" which act as buffers, opposing the formation of injurious acids. The neutralized organic substances are also more readily decomposed by microorganisms, making the nutrients available for plant growth. The soil "humus" acts as a buffer not only to changes in reaction, but in its effects upon tilth, moisture, soil temperature, and soil solution, preventing harmful extremes and making mineral soils stable for cropping purposes (168). Weir (242) claimed that by removing 40 per cent of the soil nitrogen with sodium hydroxide, and using the residual soil for vegetative experiments, approximately the same yields were obtained as from the untreated soil. However, Crowther (44) reported later that the removal of the "humus" by alkaline extraction from both a garden and a field soil reduced the productiveness over a series of crops in pot experiments. The initial increase observed in field soil was only temporary.

The amount of "humus" in the soil varies not only with depth but also with age of soil (200). Soil treatment influences the amount of "humus" in the soil, but does not change materially the ratio of the carbon to the nitrogen (96, 41). This carbon-nitrogen ratio is higher, however, in surface soil layers than in the subsoil (55, 10). Liming was found to hasten the decomposition of the soil organic matter (93, 173, 245) in peat and in mineral soils.

Without going further into a discussion of the literature on the rôle of organic matter in the soil, it is sufficient to state that it lies at the very base of our agricultural practice and soil treatment.

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